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Aus dem Kontroll-Laboratorium der Farmitalia, Como, Italien.

Über das polarographische Verhalten des Iso-Nicotinsäurehydrazids.

Von

A. Anastasi, E. Mecarelli und L. Novacic.

Mit 7 Abbildungen.

(Eingelangt am 4. August 1952.)

Wir stellten uns die Aufgabe, das Verhalten des Isonicotinsäurehydrazids an der Quecksilbertropfelektrode insbesondere in gepufferten Lösungen zu untersuchen und die Möglichkeit zu prüfen, Verunreinigungen dieser Substanz oder ihre teilweise Zersetzung festzustellen.

Für diese Untersuchungen wurde der Polarograph PO3 „Radiometer" benützt. Die Stromspannungskurven wurden mit einem Registrierinstrument aufgezeichnet. Die Potentiale sind auf die gesättigte Kalomelelektrode bezogen. Diese war über Kaliumchlorid-Agar mittels eines Hebers mit dem Meßgefäß verbunden. Die Werte $m^{2/3}$ und $t^{1/6}$, in denen m das Gewicht des in einer Sekunde abtropfenden Quecksilbers und t die Bildungszeit eines Tropfens bedeuten, wurden durch direkte Messung bestimmt. Der Sauerstoff wurde aus den Lösungen durch Einleiten von Stickstoff während zehn Minuten entfernt. In allen Fällen wurde Gelatine zur Unterdrückung des Maximums zugesetzt. Die Pufferlösungen wurden nach *Britton* und *Robinson*[1] hergestellt. Die Versuchstemperatur wurde bei 25° C konstant gehalten.

Die Untersuchungen wurden gleichzeitig auf Iso-Nicotinsäure, Pyridin, Hydrazinsulfat und Nicotinsäureamid ausgedehnt, um deren eventuell gleichartiges oder verschiedenes Verhalten feststellen zu können. Die experimentellen Daten sind in der Tabelle 1 zusammengestellt. Die Werte entsprechen 2×10^{-4}-molaren Lösungen, die Gelatine enthielten (0,008%). Diese wurden durch Verdünnen von 0,8 ml einer 0,025-m Lösung auf 100 ml unter Zusatz von 0,08 ml einer 1%igen Gelatinelösung hergestellt.

Aus den Daten der Tabelle ist zu entnehmen:

1. Die Reduktion bei p_H-Werten unter 8 führt zu schlecht definierten polarographischen Stufen, die die Tendenz haben, sich in zwei Teilstufen aufzuspalten, und die daher für analytische Zwecke nicht geeignet sind.

Tabelle 1.

p_H	Iso-Nicotinsäurehydrazid		Iso-Nicotinsäure		Pyridin		Hydrazinsulfat		Nicotinsäure	
	I_d in μA	$E^{1}/_{2}$ Volt	I_d in μA	$E^{1}/_{2}$ Volt	I_d in μA	$E^{1}/_{2}$ Volt	I_d in μA	$E^{1}/_{2}$ Volt	I_d in μA	$E^{1}/_{2}$ Volt
Puffer n. *Britton*										
2,4	4,85	— 0,63; — 0,78	2,65	— 0,89	keine Welle	—	keine Welle	—	keine Welle	—
4	5,44	— 0,75; — 0,9	2,5	— 1	„	—	„	—	„	—
5,3	5,4	— 0,86; — 1,00	2,23	— 1,08	„	—	„	—	„	—
6,5	5,3	— 1,00; — 1,1	1,97	— 1,18	„	—	„	—	„	—
7,5	4,2	— 1,08; — 1,17	1,56	— 1,3	„	—	„	—	Welle nicht meßbar	— 1,65
9,3	3,5	— 1,27	Welle nicht meßbar	— 1,55	„	—	„	—	— 1,7	— 1,68
	Anoden-Welle	— 0,05					Anoden-Welle	— 0,05		

2. Es besteht keine Ähnlichkeit zwischen dem Verhalten des Hydrazids und der Iso-Nicotinsäure. Das Halbwellenpotential der Säure verschiebt sich bei steigenden p_H-Werten gegen negative Werte, gleichzeitig nimmt die Höhe der Stufe ab, bis sie gänzlich verschwindet. Wir folgern daraus, daß nur das undissoziierte Molekül der Iso-Nicotinsäure die Elektrode dem Wasserstoff gegenüber depolarisiert.

3. Das Halbwellenpotential des Hydrazids verschiebt sich bei zunehmenden p_H-Werten gegen negativere Werte, ohne daß sich jedoch die Höhe der Stufen wesentlich ändert. Diese Reduktion ist also gänzlich verschieden von der der Iso-Nicotinsäure. Sie führt zu einer Verbindung, deren Konzentration sich mit einer Veränderung des p_H nicht ändert. Dies stimmt mit der Annahme gut überein, daß das normale Hydrazidmolekül eine direkte Reduktion erfährt.

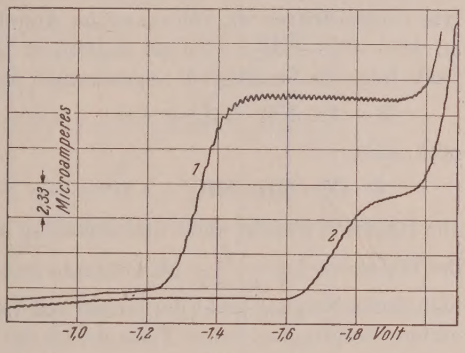


Abb. 1.
Kurve *1*: Hydrazid der Iso-Nicotinsäure, 7,5 × 10^{-4}-m.
Kurve *2*: Nicotinsäureamid, 7,5 × 10^{-4}-m.

4. Bei keinem p_H konnte eine Diffusionswelle für das Pyridin gefunden werden.

5. Das Hydrazinsulfat erzeugt eine Anodenwelle nur in alkalischer Lösung. Das Hydrazid gibt unter den gleichen Umständen eine ganz ähnliche Welle.

6. Das Nicotinsäureamid gibt bei p_H-Werten unter 8 keine polarographischen Stufen; der Grund hierfür ist die Störung seitens des Pufferwasserstoffes. Man erhält dagegen eine deutliche Stufe bei höheren p_H-Werten.

7. Bei Vergleich der Wellen des Nicotinsäureamids und des Hydrazids bei $p_H = 9{,}3$ fällt auf, daß die erste Stufe ungefähr der halben Höhe der zweiten entspricht (Abb. 1).

Annahmen über die Reaktionen an der Elektrode.

Wir haben versucht, die Zahl der an der Elektrodenreaktion in alkalischer Lösung teilnehmenden Elektronen durch Berechnung des Diffusionskoeffizienten aus dem Molvolumen mittels der Gleichung von *Stokes-Einstein* zu bestimmen:

$$D = \frac{3{,}32 \times 10^{-5}}{(V_m)^{1/3}} \text{ cm}^2 \text{ sec}^{-1}.$$

Das Molvolumen wurde direkt aus dem spezifischen Gewicht bestimmt. Setzt man den so gefundenen Diffusionskoeffizienten in die Gleichung

von *Ilkovic* ein, so ergibt sich die Teilnahme von vier Elektronen an der Reduktion. Dies stimmt gut mit der Tatsache überein, daß die Stufenhöhe des Nicotinsäureamids, an dessen Reduktion zwei Elektronen teilnehmen[2], ungefähr die Hälfte der Stufenhöhe des Hydrazids beträgt. Der Unterschied im Verhalten des Amids und des Hydrazids der Iso-Nicotinsäure scheint uns nicht begründet. In der Literatur finden sich keine Arbeiten über das polarographische Verhalten der Hydrazide. Das verschiedene Verhalten der Iso-Nicotinsäure und die Tatsache, daß das Hydrolyseprodukt eine um so kleinere Welle erzeugt, je weiter die Hydrolyse vorgeschritten ist, könnte zu der Annahme führen, daß die Reduktion an der —CO—NH—-Gruppe stattfindet. Es können daher an der Elektrode folgende Reaktionen angenommen werden[3, 4, 5]:

$$R—CO \cdot NH \cdot NH_2 + 4\,H^+ + 4\,e \rightarrow RCH_2NH \cdot NH_2 + H_2O$$

oder aber

$$R \cdot CO \cdot NH \cdot NH_2 + 4\,H^+ + 4\,e \rightarrow RCH_2OH + H_2N \cdot NH_2.$$

Die Reaktion scheint nicht umkehrbar zu sein, weil man durch Auftragen der Werte von $\log \frac{I}{I_d - I}$ als Ordinate und E als Abszisse eine Gerade erhält, deren Neigung nicht derjenigen von vier Elektronen entspricht. Eine andere Bestätigung dieser Tatsache ist die Verschiebung des Halbwellenpotentials mit steigender Konzentration von — 1,27 V für $2 \cdot 10^{-4}$-m Lösungen auf — 1,30 V für $1 \cdot 10^{-3}$-m Lösungen.

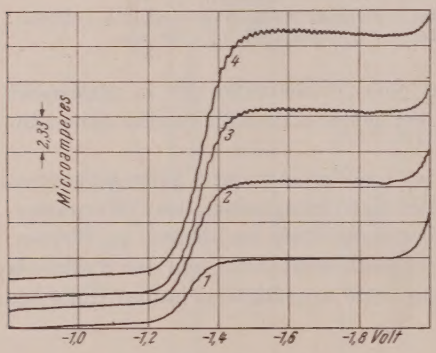


Abb. 2 Hydrazid der Iso-Nicotinsäure.
Kurve *1*: $2{,}5 \times 10^{-4}$-m.
Kurve *2*: $5{,}0 \times 10^{-4}$-m.
Kurve *3*: $7{,}5 \times 10^{-4}$-m.
Kurve *4*: $1{,}0 \times 10^{-3}$-m.

Analytische Anwendungen.

Aus unseren Untersuchungen ergibt sich, daß schwach alkalische Lösungen vom $p_H = 9$ für analytische Zwecke am besten geeignet sind. Unter diesen Umständen erhält man eine deutliche Stufe gegen die gesättigte Kalomelelektrode. Diffusionsstrom und Konzentration sind einander proportional (Abb. 2 und 3).

Es wurden viele Lösungen im Konzentrationsintervall von 10^{-4} bis 10^{-3} durch Verdünnen entsprechender Mengen der 0,025-m Stammlösung mit *Britton-Robinson*-Puffer vom $p_H = 9$ der 0,01% Gelatine enthielt, hergestellt und im Bereich von — 0,8 bis — 1,8 V untersucht. Die Resultate stimmen gut mit der chemischen Analyse überein und die

Methode wird von uns laufend zur Kontrolle gewisser Zubereitungen (Nicizina für Injektionen, in Tabletten, in Syrup) angewendet.

Die Bestimmungen können auch in Natriumhydroxyd-Natriumacetat-Puffer ausgeführt werden. Wir ziehen diesen für Serienanalysen der leichteren Herstellbarkeit wegen vor. In diesem Falle ist das Halbwellenpotential negativer (— 1,33 V anstatt — 1,27 V) und verschiebt sich um 0,03 V bei zunehmender Konzentration wie oben.

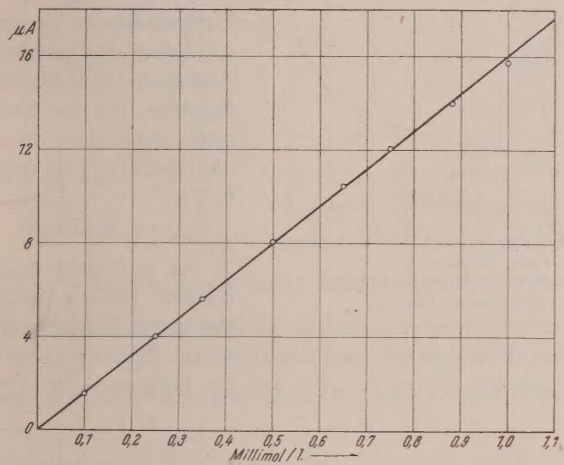


Abb. 3. Beziehung zwischen Diffusionsstrom und Konzentration des Iso-Nicotinsäurehydrazids.

C Millimol/l	I_d µA	I_d/C µA/millimol/l	C Millimol/l	I_d µA	I_d/C µA/millimol/l
0,1	1,6	16	0,6	9,5	15,83
0,2	3,2	16	0,7	11,1	15,85
0,3	4,75	15,83	0,8	12,7	15,87
0,4	6,3	15,75	0,9	14,3	15,88
0,5	8	16	1	15,9	15,9

Die Feststellung von Zersetzungsprodukten.

Wie schon erwähnt, sinkt der Diffusionsstrom bei eintretender Hydrolyse des Hydrazids. Diese Tatsache ist von großem Interesse für die Prüfung der Haltbarkeit der damit hergestellten Zubereitungen.

Iso-Nicotinsäure.

Untersucht man Lösungen, die zunehmend stärker hydrolysiert sind, so kann man feststellen:

1. Bei hohen p_H-Werten erfolgt in Acetatpuffer eine Erniedrigung der Stufe, die dem Ausmaß der Hydrolyse proportional ist, da in diesem Falle

keine Reduktion der gebildeten Iso-Nicotinsäure stattfindet (Abb. 4). Im *Britton*puffer erzeugt dagegen die Iso-Nicotinsäure eine Reduktionsstufe, die abgesehen davon, daß sie undeutlich und nicht meßbar ist, auch die Auswertung der Hydrazidstufe verhindert.

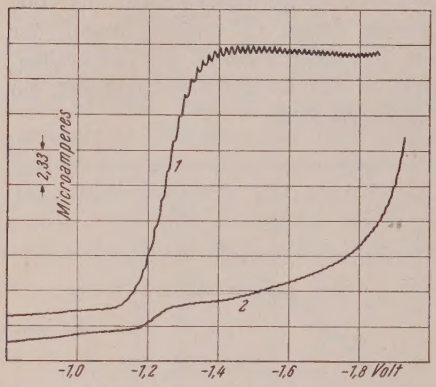
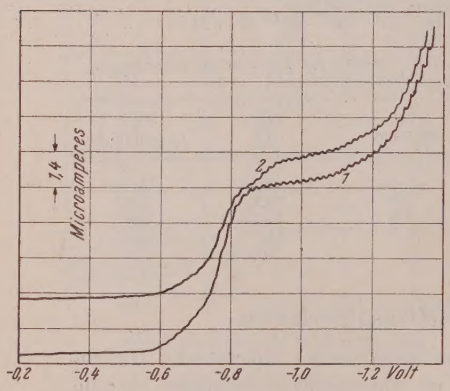


Abb. 4. Iso-Nicotinsäurehydrazid, 10^{-3}-m, in Acetatpuffer bei $p_H = 9$.
Kurve *1*: Nicht hydrolysierte Lösung.
Kurve *2*: Hydrolysierte Lösung.

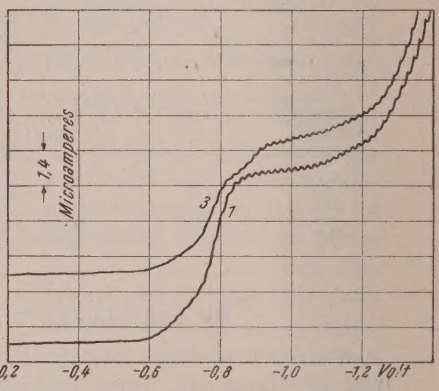
2. Im *Britton*puffer vom $p_H = 2{,}0$—$2{,}5$, in dem die Hydrazidstufe wenig deutlich ist, kann man parallel mit dem Absinken der Hydrazidstufe die Zunahme der Iso-Nicotinsäurestufe feststellen, die sofort auf die Hydrazidstufe folgt (Abb. 5, 6, 7).

Hydrazin.

Es war nicht möglich, eine direkte polarographische Methode zum Nachweis des Hydrazins zu finden. Dies deshalb, weil das Hydrazid (Nicizina) eine anodische Welle beim gleichen Potential gibt wie das Hydrazin. Die Oxydations-



5a



6a

Abb. 5a u. 6a. Iso-Nicotinsäurehydrazid, $2{,}5 \times 10^{-4}$-m. in *Britton-Robinson*-Puffer bei $p_H = 2{,}3$.
Kurve *1*: Nicht hydrolysierte Lösung.
Kurve *2*: Schwach hydrolysierte Lösung.
Kurve *3*: Stärker hydrolysierte Lösung.

stufen der beiden Substanzen, die schon von anderen beobachtet wurden[6], erscheinen bei höherem p_H besser definiert. Das Hydrazin als Verunreinigung kann man aber sehr gut auf chemischem Wege be-

stimmen[7]. Das Hydrazid reagiert quantitativ mit salpetriger Säure nach folgender Gleichung:

$$RCO—NH—NH_2 + HNO_2 \rightarrow RCON_3 + 2\,H_2O$$

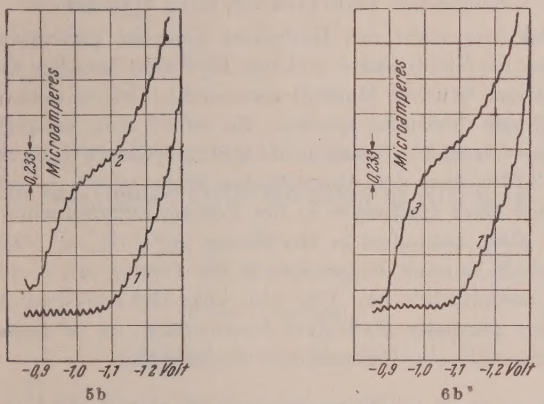


Abb. 5 b. u. 6 b. Die Stufen der Abb. 5 a und 6 a, mit größerer Empfindlichkeit aufgenommen.

während das Hydrazin die doppelte Menge salpetriger Säure verbraucht;

$$H_2N \cdot NH_2 + 2\,HNO_2 \rightarrow 3\,N + 3\,H_2O + NO.$$

Titriert man also eine Substanzmenge oder eine Lösung, deren ursprüng-

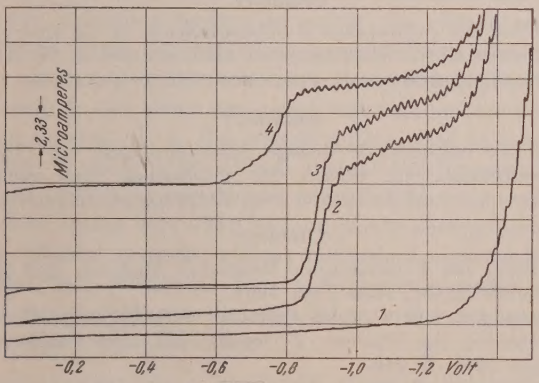


Abb. 7. Lösungen in *Britton-Robinson*-Puffer bei pH = 2,3.
Kurve *1*: Grundelektrolyt.
Kurve *2*: Hydrazid, 10^{-3}-m, vollständig hydrolysiert.
Kurve *3*: Iso-Nicotinsäure. 10^{-3}-m.
Kurve *4*: Hydrazid, $2{,}5 \times 10^{-4}$-m, nicht hydrolysiert.

lichen Gehalt man kennt, so kann man den Hydrazingehalt nach folgender Formel berechnen:

$$\% \text{ Hydrazin} = \frac{(C - a_1) \cdot 100}{a_2 - a_1}$$

In dieser Gleichung bedeuten:

C ..... Verbrauch an ml für 100 g Substanz,
a_1 ..... Theoretischer Verbrauch für 100 g reine Substanz,
a_2 ..... Theoretischer Verbrauch für 100 g Hydrazin.

Da das Molekulargewicht des Hydrazins klein ist gegenüber dem des Hydrazids der Iso-Nicotinsäure und das Hydrazin überdies die doppelte Menge salpetriger Säure je Molekül verbraucht, können auch sehr kleine Mengen Hydrazin bestimmt werden. So erhält man beispielsweise für eine Mischung von 99,5% Nicizina und 0,5% Hydrazin einen scheinbaren Gehalt, der 3,77% über dem theoretischen liegt.

Das infolge einer Hydrolyse in der Lösung aufscheinende Hydrazin zersetzt sich aber, besonders in Berührung mit Luft, in Stickstoff und Wasser[8], weshalb es nach einiger Zeit in der Lösung mit dieser Methode nicht mehr nachweisbar ist. Um also eine Gehaltsverminderung der Lösung infolge partieller Hydrolyse festzustellen, ist es angezeigt, eine direkte polarographische Bestimmung vorzunehmen.

Zusammenfassung.

Es wird eine Methode der polarographischen Bestimmung des Hydrazids der Iso-Nicotinsäure beschrieben. Es werden Angaben über die Feststellung von dessen Zersetzungsprodukten in pharmazeutischen Zubereitungen gemacht.

Summary.

A method for the polarographic determination of the hydrazide of isonicotinic acid is described. Statements are made concerning the establishment of its decomposition products in pharmaceutical preparations.

Résumé.

On décrit une méthode de dosage polarographique de l'hydrazide de l'acide iso-nicotinique. On donne des indications pour rechercher des produits de décomposition de celui-ci dans des préparations pharmaceutiques.

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Institute of Medical Chemistry, University of Uppsala, Sweden.

On the Dumas Determination of Nitrogen.

By

W. Kirsten.

With 7 figures.

(Received August 11, 1952.)

At first sight the *Dumas* nitrogen determination appears to be a quite simple thing: The sample is burned to carbon dioxide and water by heating with copper oxide. The nitrogen is liberated as elementary nitrogen or as nitrogen oxides. The latter are reduced by the layer of metallic copper in the combustion tube. Halogens and sulphur are converted to acidic oxides or hydrides or retained in the tube. All liberated compounds are absorbed in the potassium hydroxide except the elementary nitrogen which is collected above the potassium hydroxide and measured there.

There are, however, many other reactions which play important rôles in the method, and which can cause considerable errors if they are not taken into account. E. g. the present author tried to analyze silver nitrate according to the ordinary *Dumas* method[1] but frequently obtained very low results, which appeared quite inexplicable.

As the present author very often had samples to analyze which showed difficulties in different respects, various methods were tried in order to solve such analytical problems. The investigations carried out in the course of these experiments are discussed below, and a new method based upon the investigations is described.

Combustion.

In the ordinary *Dumas* nitrogen determination procedure[1] the combustion conditions are much less satisfactory than in the carbon and hydrogen determination procedure, essentially depending upon the facts[1] that no free oxygen is present, the combustion being carried out in carbon dioxide which is a reaction product of the combustion and therefore must be expected to have an adverse effect: and[2] that the combustion

is carried out at a lower temperature. There are essentially two types of incomplete combustion which occur in the determination: incomplete combustion in the gas-phase which allows unburned gases like methane to pass through the tube and causes high results; and incomplete combustion in the solid phase by formation of nitrogen-containing charcoal, causing low results. Experiments with a nitrogen determination apparatus with nickel oxide filling[2, 3] indicate, however, that the latter type of incomplete combustion does not occur as often as is generally believed.

According to *Kao* and *Woodland*[4] conditions are very favourable for the combustion of carbon monoxide to carbon dioxide and of hydrogen to water in the ordinary *Dumas* procedure. Errors from these gases can therefore hardly be expected. In earlier work[2, 3, 5, 6, 7, 8, 9] it has been stated that different types of compounds such as aliphatic long-chain compounds and compounds with condensed ring systems, especially these with angular methyl groups, are very difficult to burn. This has generally been ascribed to the fact that considerable amounts of methane are formed during the cracking of these compounds, and that the methane is the incombustible compound. *Van Meter*, *Bailey* and *Brodie*[10] also found considerable amounts of hydrocarbons, mostly methane, when they analyzed the gasmixture obtained in an ordinary *Dumas* combustion of shale oil. Experiments carried out by the present author indicate that a rapid and complete combustion of methane can only be obtained at temperatures above that at which auto-decomposition of methane begins, which seems to lie between 700 and 800° C[11].

Van Meter, *Bailey* and *Brodie*[10] found nitric oxide in the combustion gases, which can only be explained by the fact that incompletely burned nitrogen-containing gases have passed through the copper layer and have been oxidized to nitric oxide in the succeeding layer of copper oxide. This shows that in the ordinary *Dumas* nitrogen determination, using copper oxide, the combustion should be conducted so that complete combustion of all gaseous nitrogen compounds is achieved before the gases enter into the layer of metallic copper. This is provided for in a very ingenious manner in the method described by *Zimmermann*[12], who leads the combustion gases twice over parts of the tube-filling before they enter into the layer of copper. This also improves the overall oxidation capacity of the tube. When nickel oxide is used this provision is not necessary for reasons dealt with below.

Many experiments have been made to avoid the error ascribed to incomplete combustion caused by formation of charcoal. *Friedrich*[13] and *Roth*[1] state that reliable results cannot be expected when oxygen-liberating compounds are added to the samples. The present author also obtained unreliable results in similar experiments. *Brancone* and

Fulmor[14] increased the temperature of the sample-heater to 900° C and reported good results. *Unterzaucher*[15, 16] adds oxygen derived from hydrogen peroxide to the carbon dioxide and reports complete burning of refractory compounds. *Alford*[17] reported on a similar procedure. The present author[18, 19] obtained complete burning of refractory compounds using the reaction $C + CO_2 = 2\,CO$ which takes place rapidly at temperatures around 1050° C.

Other Reactions.

The states of the gas equilibria in the *Dumas* nitrogen determination have been investigated by *Kao* and *Woodland*[4]. From their results it can be seen that the errors from interfering gases should be very small at lower temperatures, provided that the combustion is complete. At temperatures around 1000° C dissociation of carbon dioxide takes place[2, 4] necessitating the use of hopcalite[2]. The experiments of the present author described below show that nitrogen oxides are retained by copper oxide and nickel oxide when free oxygen is present in the combustion tube. As cupric oxide liberates oxygen when heated to higher temperatures[20] the conditions for retention of nitrogen oxides often exist in the ordinary *Dumas* procedure when the sample-heater or the combustion furnace are heated too strongly. It seems, therefore, that low results which have been ascribed to the formation of charcoal in reality have often been caused by the retention of nitrogen oxides. Many analysts heat their used copper oxide — which is partly reduced — in carbon dioxide for regeneration. This treatment converts considerable amounts of the cupric oxide to cuprous oxide, diminishing the risk of oxygen-liberation. Nickelous oxide only begins to liberate oxygen at much higher temperatures[21].

In the ordinary *Dumas* procedure a layer of metallic copper is used in the middle of the combustion tube. This is necessary for the retention of liberated oxygen and for the reduction of nitrogen oxides split off from the samples or formed in the combustion of the gases by contact with cupric oxide. When nickel oxide alone is used, formation of nitrogen oxides by combustion cannot take place, since any oxidation results in the formation of metallic nickel, which immediately reduces the nitrogen oxides formed. Therefore only the nitrogen oxides which are split off from the samples have to be reduced. For this purpose the metallic nickel formed by the combustion of the samples, or even the metal of the capsule used in the procedure described below, is completely satisfactory.

Blanks.

Blanks have generally been considered to arise from two causes: air adsorption on the copper oxide of the temporary filling, and impure

carbon dioxide. *Trautz*[22] found the air adsorption blank in an ordinary micro *Dumas* determination to have a value of 0.006 ml. *Pagel* and *Oita*[23] stated that the air adsorption error should not rise above 0.001 ml. The present author[2, 3], using regenerated copper oxide, found values near to those of *Trautz*, and *Wallberg-Olausson*[24], with new copper oxide, found blanks nearer to those of *Pagel* and *Oita*, but stated that higher and somewhat irregular blanks were obtained with regenerated copper oxide. The difference can probably mainly be explained by the differing surface activities of different specimens of copper oxide. That such differences play an important rôle is also shown by the differing reactivities of different specimens of the metal oxides towards nitrogen oxides as described below.

Gonick, *Tunicliff*, *Peters*, *Lykken* and *Zahn*[15] state that high nitrogen results can be caused by the release of nitrogen adsorbed on copper oxide when the latter is reduced to the metallic state by burning the sample. As these authors add oxygen to the combustion gases in their nitrogen determination method and operate at 750° C all conditions for the retention of nitrogen oxides in the copper oxide seem to be present. It is, therefore, probable, that the nitrogen released from their copper oxide was not adsorbed but was chemically bound. The experiments related below also show that copper oxide and nickel oxide for the *Dumas* nitrogen determination should not be prepared by heating the nitrates. Oxides which contain chemically bound nitrogen derived from the preparation or from earlier analyses can, however, be purified by reduction with hydrogen and subsequent reoxidation.

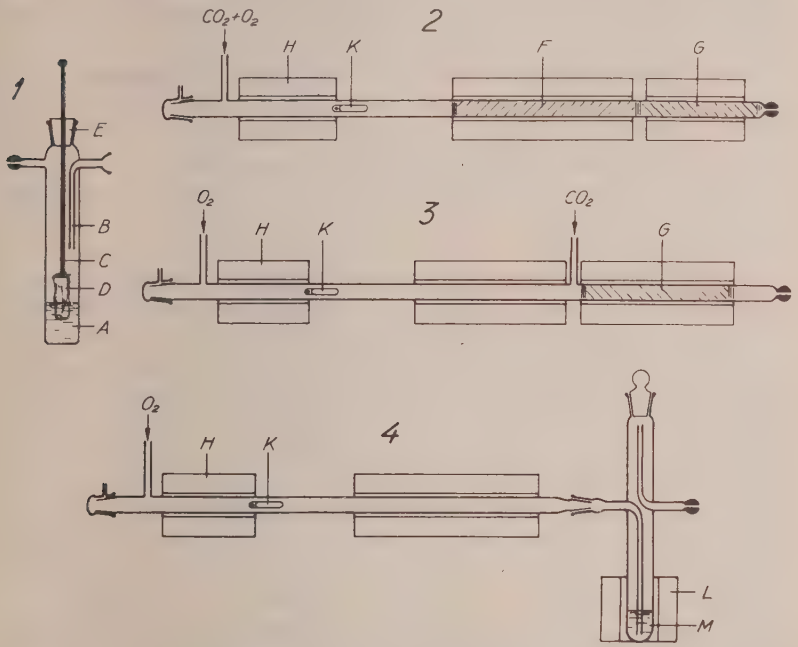
Blanks from carbon dioxide are very general and many different generators for pure carbon dioxide have been described. The present author has found the use of dry-ice in a thermos-flask very convenient[26, 27]. The source of the dry-ice seems, however, to be important, and poor results with dry-ice have been reported[28]. Excellent carbon dioxide can often be obtained in cylinders[29]. Care should be taken that the connection to the apparatus is made of material which does not allow diffusion of nitrogen through its walls. As one filling has a very long life-time the blank remains constant over a long period of time. This is especially useful in work of high accuracy and in trace-determination methods.

It seems that blanks which have been ascribed to impurities in the carbon dioxide or to air adsorption have often been caused by the liberation of small amounts of oxygen due to excessive temperatures in the combustion furnace[23].

The fact that rubber tubing and rubber stoppers cause blanks due to diffusion of nitrogen through them has been pointed out by several authors[2, 3, 22, 30, 31, 32].

Experimental Part.

Recently *Unterzaucher*[15, 16] published a method for the determination of nitrogen, in which oxygen developed from hydrogen peroxide was used for the burning of the samples. The method has been tried in the author's laboratory. *Unterzaucher* did not give details such as temperatures, gas speeds etc. The vessel shown in Figure 1 was used for the



Figures 1—4. *A* = 30% Hydrogen peroxide, *B* = Inlet tube, *C* = Movable glass rod, *D* = Platinum sheet, *E* = Rubber stopper sealed into joint with *Krönig's* glass cement. Inner bore lubricated with glycerol, *F* = Copper oxide, *G* = Metallic copper, *H* = Sample heater, *K* = Boat with sample, *L* = Heating block, *M* = Boiling sulphur.

development of the oxygen. It allowed a limited regulation of the speed of the gas-development by raising or lowering the glass-rod C, on which the platinum was fixed, so that it could be immersed more or less in the hydrogen peroxide. The tube shown in Figure 2 was used for the determinations. A gas-speed of 5 ml of carbon dioxide and 2 ml of oxygen was used. The copper oxide furnace was kept at a temperature of 700° C and the copper furnace at 500° C. *Unterzaucher*'s statement that copper oxide does not reduce carbon dioxide was confirmed and good results were obtained in the first few analyses. However, with every analysis the necessary sweeping-time increased and quite soon this time reached

several hours, so that no more analyses could be carried out. When a new tube-filling was prepared with new copper oxide the same thing happened. After a few analyses it was no longer possible to sweep out the nitrogen. Microbubbles could not be obtained, and large amounts of nitrogen remained in the tube. In order to find out where the nitrogen was retained the temperatures of the furnaces were changed during the sweeping. Changes in the temperature of the copper filling between 200 and 800° C had no influence upon the size of the bubbles. When the temperature of the copper oxide furnace was raised the size of the bubbles increased, and at lower temperatures microbubbles were obtained though large amounts of the nitrogen still remained in the tube. It seemed, therefore, that the nitrogen was retained in the copper oxide. The same experiments were then repeated with nickel oxide and nickel instead of copper oxide and copper. In this case large amounts of nitrogen were retained even in the first analysis. The nitrogen was retained in the nickel oxide.

As the first analyses using copper oxide usually were good, it was presumed that the copper oxide might contain some impurity such as calcium sulfate or barium sulfate which could be reduced to the carbonate during the analyses, and that this carbonate could retain nitrogen oxides. Highly purified copper oxide and nickel oxide were, therefore, prepared and used. They gave low results even in the first analyses. It was then assumed that the reaction products formed between the quartz tube and the metal oxide during the analyses might cause the errors. Copper tubes and nickel tubes were therefore placed inside the combustion-tube and the gases led through in such a manner that the whole combustion took place inside the metal tubes. The results were the same. Finally an empty-tube combustion was tried as shown in Figure 3. The sample was burned in an oxygen flow at 1000° C. Carbon dioxide was then blown in from the side, and the gases were swept through the copper into the nitrometer. In this case also low results were obtained after a few good analyses, though the number of satisfactory analyses which could be carried out with one filling was greater than with the tube fillings used before. As it seemed that the copper oxide would always retain nitrogen, another material was sought for the reduction of the gases in the empty-tube combustion. Figure 4 shows an arrangement in which the combustion gases were led through boiling sulphur after the oxidation. Satisfactory microbubbles could not, however, be obtained with this arrangement. It is possible that the interfering gases could have been taken out with a cold trap. The author overlooked this possibility during the investigation. In addition the purity of the sulphur used may not have been satisfactory.

During the investigation attempts were made to prepare pure nickel oxide and pure copper oxide by heating the nitrates. Extremely high nitrogen blanks were obtained in the combustion of nitrogen-free compounds when these oxides were used in an ordinary *Dumas* combustion without oxygen. Copper oxide and nickel oxide which had been used in a *Dumas* combustion with oxygen were found to behave similarly. When these oxides were reduced with hydrogen and reoxidized with oxygen high blanks were no longer obtained. This experiment was repeated with nickel oxide which had been used as catalyst in the carbon and hydrogen determination[9]. It contained very large amounts of nitrogen and gave high blanks. When carrying out analyses without a scrubber in the carbon and hydrogen method the author had often obtained very slightly high carbon results in the analysis of nitrogen-free compounds. Consequently the scrubber was also used when nitrogen-free compounds had to be analyzed. These slightly high results possibly result from the reduction of nitrogen in the catalyst by the reducing gases of the samples.

In order to find out if nitrogen retention errors also would occur in the ordinary *Dumas* determination method if oxygen was not added as free oxygen, but was liberated from the cupric oxide, the following experiment was carried out; Copper oxide was reduced with hydrogen and reoxidized by heating in oxygen at 500° C for 24 hours. A combustion tube was then filled with this oxide in the ordinary manner[1] except that a layer of copper and a small layer of hopcalite were added at the end of the tube. The filling was then heated to 800° C. Good microbubbles were obtained, but when analyses were carried out with combustion at 800° C, very low and inconsistent results and unsatisfactory microbubbles were obtained.

It seems that from all these experiments the following conclusions can be drawn: There exist compounds of oxygen and nitrogen with nickel or copper respectively which are stable in atmopheres of oxygen and carbon dioxide at very high temperatures. Only at temperatures around 800° C does begin very slow decomposition. The compounds are formed either by heating the metal nitrates or by leading nitrogen oxides together with oxygen over the red-hot oxides. Finely divided oxides, or copper oxide wire which has been reduced and oxidized several times, take up the nitrogen very quickly, while new copper oxide wire reacts very slowly, probably because of differences in surface activity. Hydrogen at red heat reduces the compounds to the nitrogen-free metals. The fact that copper oxide which has been prepared by heating the nitrate can contain up to four or five times its own volume of nitrogen has already been observed by *Richards*[33]. *Sabatier* and *Senderens*[34] report the formation of a compound "nitro-copper" when nitrogen dioxide is led over finely divided copper. *Park* and *Partington*[35, 36] and *Klemenc* and

Schroth[37] explain this compound as a product of the adsorption of nitrogen dioxide on cuprous oxide. Perhaps the reactions described above, which take place at high temperatures, are of a similar kind.

Because of these results it was difficult for the present author to understand how *Unterzaucher*[15, 16] obtained satisfactory results with his method. The author has since learned[38] however, that the amount of oxygen which *Unterzaucher* adds in his procedure is so small, that the first gases volatilized from the sample immediately consume all oxygen, creating an oxygen-free atmosphere in the combustion tube. The author was also

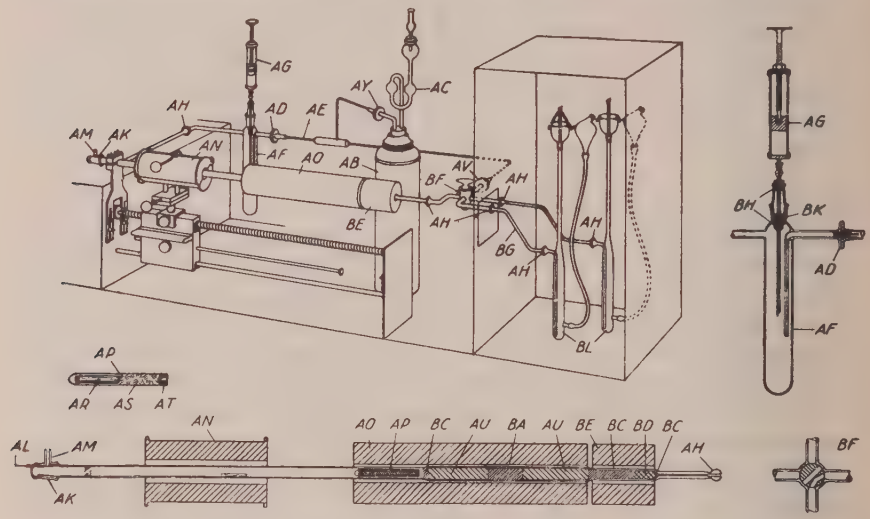


Fig. 5.

surprised by the results recently published by *Alford*[17]. Though the nitrogen retention error can be seen immediately in the results published by this author (the centre of gravity of the errors in his tables lying around — 0.06%) the present author would have expected much larger errors judging from his own results. It may be that the freshly prepared oxygen used in the present author's experiments was more reactive and increased the error, whereas in *Alford*'s experiments oxygen kept in a tank was used.

Following on these experiments it seemed to the present author that a nitrogen determination method using oxygen for the combustion should give good results provided that the oxygen is prevented from entering into the tube-filling. The apparatus shown in Figure 5 was therefore constructed. Preparations of the oxygen by catalytic decomposition of hydrogen peroxide was inconvenient, since the reaction is exo-

thermic and is strongly accelerated at higher temperatures. A careful control of the temperature of the vessel is therefore necessary in order to keep the gas-development within the desired limits. As an alternative the apparatus shown in Figure 5 was developed. The syringe contained

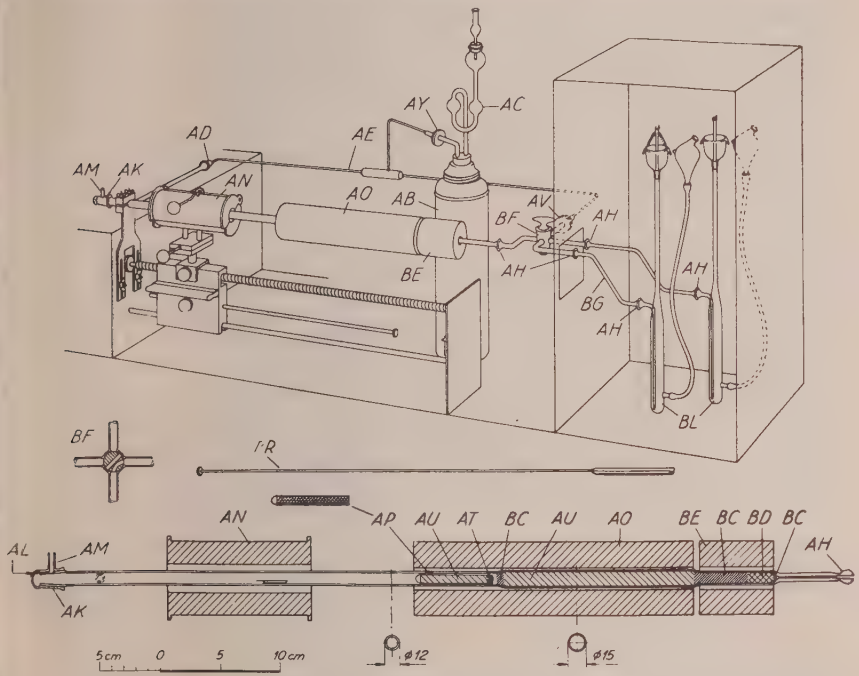


Fig. 6.

Figures 5 and 6. *AB* = Thermos flask, *AC* = Mercury valve tube as used in fermentation processes, *AD* = Joint with inserted capillary which regulates carbon dioxide flow, *AE* = Flexible copper tubing, *AF* = Vessel containing solution of one part by volume of concentrated sulfuric acid in three parts of 30% hydrogen peroxide, *AG* = Syringe containing 50% solution of sodium permanganate, *AH* = Ball and socket ground joints, *AK* = Joint for closing combustion tube, *AL* = Score in ground joint for passage of sweeping gas, *AM* = Side-tube for passage of sweeping gas, *AN* = Sample-heater, *AO* = Combustion furnace, *AP* = Capsule, *AR* = Roll of copper oxide wire gauze, *AS* = Metallic copper, *AT* = Plug for closing capsule, *AU* = Nickel oxide, *AV* = Flat ground joint with inserted capillary for regulation of gas flow in reverse sweeping, *AY* = Flat ground metal joints, *BA* = Metallic nickel, *BC* = Asbestos, *BD* = Hopcalite, *BE* = Heating block for hopcalite, *BF* = Special four-way stopcock, *BG* = Glass capillaries, *BH* = Rubber stoppers sealed into joints with *Krönig's* glass cement. *BK* = Tapered ground joint lubricated with silicone grease, *BL* = Nitrometers, *BR* = Long handled spatula capsule.

a concentrated solution of sodium permanganate in water and the vessel a mixture of hydrogen peroxide and concentrated sulfuric acid. Both solutions are fairly stable, and when a drop of sodium permanganate is allowed to fall into the hydrogen peroxide a stoichiometrically determined amount of oxygen is developed.

A capsule of stainless steel wire gauze with a small copper oxide roll and copper was used as temporary filling. The sample was weighed into a platinum boat and inserted into the combustion tube in the same manner as in the carbon and hydrogen determination. After sweeping, the tube was closed and connected with the nitrometer. Two or three drops of sodium permanganate were then allowed to fall into the hydrogen peroxide, the sample-heater was drawn over the tube and the motor was switched on. The copper in the capsule immediately reduced all nitrogen oxides formed and prevented all oxygen from entering into the main combustion filling. The efficiency of the copper filling could be controlled after the analyses by inspecting the remaining unoxidized copper. It was found that the copper seemed to remove all oxygen instantly. A space with partly oxidized copper was never found and sharp frontiers always occurred between the copper oxide and the copper in the capsule. The apparatus was in use in the author's laboratory for about six months, good results being obtained. Occasionally when graphite like compounds were analyzed low results were, however, obtained, and in such cases the succeeding analyses generally gave high results. This was investigated, and it was found, that all oxygen had not been consumed by the refractory compounds, and nitrogen oxides had been absorbed in the small roll of copper oxide in the capsule and reduced out again by the gases produced in the next analysis. It was therefore found necessary to add the oxygen slowly in these cases. Further experiments also showed that the small roll of copper oxide could absorb up to 0.1 ml of nitrogen in one analysis if a very large amount of oxygen was added, and if the analyst delayed the burning of the sample until the whole space around the boat was filled with oxygen. The aim was therefore to avoid all oxygen and to burn the sample using only the reaction $C + CO_2 = 2\,CO$. Rapid and easy burning of all kinds of compounds was obtained without addition of oxygen when the sample-heater was kept at a temperature of 1050° C[18]. As the copper oxide in the capsule melts at this temperature the capsule was filled with nickel oxide. It was also found, that the capsule of stainless steel wire gauze was not suitable at these high temperatures. It soon became brittle, and high results were obtained which probably were due to liberation of nitrogen from nitrides in the alloy. Capsules of perforated nickel were therefore tried. They gave excellent results but could not be used for more than ten analyses because they then became too brittle. Capsules made of nichrome, monel and inconel were then tried. They endured the temperatures well but liberated large amounts of nitrogen during the analyses. An alloy of pure cobalt and nickel may perhaps be the most suitable metal for the capsule. The author has, however, not been able to obtain such a material and capsules of pure nickel are therefore used. When

the spatula BR in Figure 6 is used, the capsule can be handled so cautiously that it can be used for up to 20 analyses*.

Practical Conclusions.

Copper oxide is not a good combustion catalyst for the *Dumas* nitrogen determination. When low temperatures are used incomplete combustion is obtained; when high temperatures are used oxygen is liberated and nitrogen oxides are retained. Nickel oxide[2, 3, 18, 19, 20, 24, 39] offers several advantages: It allows the use of temperatures over 1000° C and thus secures a complete combustion; and as nickel oxide is a stable compound which does not liberate oxygen it eliminates the risk of errors from retention of nitrogen oxides.

When very accurate results are desired the use of free oxygen or oxygen-liberating compounds for the burning should be avoided. Mixing the samples with copper oxide and heating to temperatures between 650 and 700° C seems to give satisfactory results with most compounds[28]. When the content of cuprous oxide in the copper oxide used for the temporary filling is high, the use of higher temperatures seems to be useful. But the burning method using the reaction $C + CO_2 = 2\,CO$ at high temperatures without catalyst is more accurate, of more universal use, easier to handle, avoids air adsorption blanks and obviates the need for a nickel-layer in the combustion tube. Since in addition the adsorption of water can easily be avoided, this method should be useful in the simultaneous determination of nitrogen and hydrogen described by *Wurzschmitt*[38]**.

Method Proposed.

The sample is burned in carbon dioxide at 1050° C. A capsule of perforated nickel plate filled with granulated nickel oxide is used as temporary filling. The capsule is introduced into the hot furnace followed by the sample, and the adsorbed gases are swept out using a reverse flushing system[40]. Granulated nickel oxide kept at a temperature of 1000° C is used as oxidation catalyst, and hopcalite is used for the recombination of dissociated carbon dioxide.

* It was recently found that capsules made of inconel sheet can be used after removing their nitrogen content by heating them in coal gas to 1100° C during a few hours. Such capsules have a very long life-time.

** Even graphite was found to be burned quantitatively when covered with ca. 20 times its weight of vanadium pentoxide. The correctness of the results could, however, not yet be controlled because of the lack of a suitable standard. The boat should be placed inside a small quartz tube in order to avoid contamination of the combustion tube. Vanadium pentoxide does not liberate oxygen at the temperatures used. It has earlier been used by *Downing*[41].

Reagents.

Nickel oxide: 1 kg C. P. nickel oxide is mixed with 150 g C. P. $NiSO_4 \cdot 7\,H_2O$. Water is added until a pulp is obtained. This is dried at 150° C and the resulting cake is crushed in a mortar. The powder is sieved off and the grains between 10 and 30 mesh are heated to 1000° C in a stream of carbon dioxide or oxygen for 40 minutes. After cooling, the nickel oxide is ready for use. If the reagents used contained nitrogen, the nickel oxide will give blanks in the combustion of nitrogen-free compounds. In this case it should be reduced with hydrogen and reoxidized. The reduction, and more especially the reoxidation, should be carried out very cautiously in order to avoid sintering of the oxide. It is most convenient to mix the used gas with large amounts of carbon dioxide by leading it through a bottle containing dry ice. The reaction should be watched carefully so that no high temperatures will be obtained.

Hopcalite: Ordinary commercial hopcalite was found satisfactory.

Asbestos or *quartz-wool:* C. P. grade.

Potassium hydroxide: 50% solution in water. If one drop of *iso*-amylalcohol is added to 100 ml of the solution[23] the usual treatment with barium hydroxide[1] is not necessary.

Carbon disulphide: C. P. quality.

Carbon dioxide: From cylinder, dry ice or other source.

Adjusting the Apparatus.

The apparatus is mounted as shown in Figures 6 and 7. The sample-heater is adjusted to a temperature of 1050° C, the combustion furnace to 1000° C and the hopcalite furnace to 100–150° C. The length of the sample-heater should be at least 120 mm, so that the boat with the sample is still in the hot zone when the heater has finished its trave to the combustion-furnace. Capillary AD and the mercury-levels in the pressure regulator and the nitrometers are adjusted so that a carbon dioxide stream of about 3 ml/min. passes into each nitrometer. (The flow-rate of 10 ml/min. given in the Preliminary Report[18] can be used only in the analysis of non-volatile compounds.) If bubbles tend to stick to the mercury a few drops of carbon disulphide are pipetted into the levelling bulb, dispersed by shaking and drawn into the nitrometer. A few minutes later all sticking will cease. Capillary AV, which regulates the flow-rate of the reverse sweeping, is adjusted to allow a gas-flow of about 15–20 ml/min. The combustion tube is filled as shown in the Figure. The filling should not oppose an appreciable resistance to the gas-flow. When the combustion tube has to be refilled it is generally not necessary to renew the hopcalite-filling because it is not spent by the reactions in the tube. The part of the combustion tube inside the combustion furnace is

preferably made of opaque quartz, which prevents undue heating of the sample and the joint AK by radiation inside the quartz walls. The moving speed of the sample-heater is adjusted so that a time of 18 to 20 minutes is used for the travel to the combustion furnace. Higher speeds should not be used if volatile compounds containing much carbon are to be analyzed, because the very fast volatilisation of a large sample would cause methane to pass through the tube into the nitrometer. When only non volatile compounds are analyzed, or in microanalysis, considerably

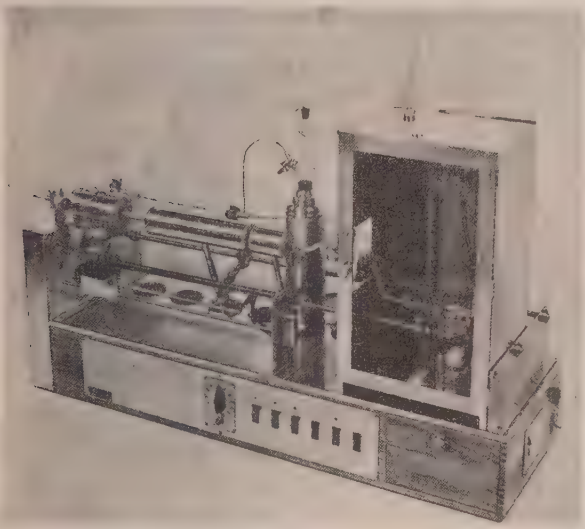


Fig. 7. Photograph of Apparatus (Courtesy Norstedt & Söner, Stockholm).

higher speeds can be used. The sample-heater should stop at a distance of 5–10 mm from the combustion furnace, as overheating with resulting burn-out could occur if the furnaces were allowed to stand close together for a longer period of time. All ground joints are connected using silicone grease lubricant, with the exception of the connections between the nitrometers and the fourway stopcock which are sealed together with *Krönig*'s glass cement. The thermos flask should be filled with dry-ice a day before the apparatus is to be used, and the stopcock should be turned so that the carbon dioxide passes in reverse through the combustion tube and out. The hopcalite is heated first to a somewhat higher temperature — about 250° C — in order to burn away adsorbed impurities, and then allowed to fall to working temperature. A few blank determinations without sample and with a nitrogen-free compound are carried out then

according to the procedure described below, in order to determine the size of the blank and to control the purity of the nickel oxide.

Procedure.

The apparatus is heated to working temperature while the gas-flow is passing in reverse through the combustion tube and out. Joint AK is closed and the combustion tube is connected first with one and then with the second nitrometer so that all connections are flushed out with carbon dioxide. The size of the microbubbles is then observed. AK is then opened again and BF turned so that the gas-flow passes in reverse. The capsule is filled with nickel oxide and introduced into the combustion furnace using spatula BR. The sample, which has been weighed into a

Table I.

No.	Substance	Weight of Sample mg	Temperature ° C	Pressure mm Hg	N_2 Corrected ml	N Found %	N Calcd %
1	p-Nitrobenzoic acid	16.10	28	752	1.208	8.44	8.38
2	Taurine	13.10	28	752	1.302	11.18	11.20
3	Cyanacetamide°	4.51	28	752	1.333	33.25	33.33
4	p-Nitrobenzoic acid	19.52	29	758	1.455	8.42	8.38
5	Taurine	17.42	29	758	1.726	11.19	11.20
6	Benzamide	17.55	30	758	1.811	11.62	11.57
7	3-Nitrophthalic acid	21.40	31	758	1.275	6.69	6.64
8	Cystine N. B. S.*	16.64	30	758	1.721	11.65	11.66
9	p-Bromoacetanilide*	19.65	30	758	1.136	6.51	6.54
10	Behenamide°	21.88	30	758	0.799	4.11	4.13
11	p-Nitrobenzoic acid	20.86	31	759	1.543	8.31	8.38
12	Taurine	16.91	31	759	1.682	11.18	11.20
13	Benzamide	17.64	30	760	1.808	11.57	11.57
14	Taurine	17.60	29	757	1.751	11.22	11.20
15	p-Nitrobenzoic acid	18.41	31	760	1.385	8.46	8.38
16	Behenamide°	21.87	30	760	0.799	4.12	4.13
17	Taurine	17.94	32	759	1.794	11.20	11.20
18	Benzamide	18.33	31	759	1.873	11.49	11.57
19	3-Nitrophthalic acid	20.71	31	757	1.220	6.60	6.64
20	Cystine N. B. S.*	17.71	30	757	1.844	11.71	11.66

All analyses were carried out in strict succession except that after analysis 13 the apparatus was accidentally destroyed and after the reconstruction four test analyses were carried out before analysis 14 in order to adjust the apparatus to the proper working conditions.

Standard deviation $\sigma = 0.045\%$.

The compounds marked° were kindly given to us by *E. Stenhagen*, those marked * by *J. A. Kuck*, American Cyanamid Comp., Stamford, Conn., U. S. A. The others were commercially available products.

platinum boat is now introduced and placed at a distance of about 9–10 cm from the combustion furnace. Joint AK is put on so that the carbon dioxide passes out through AM. After 3–5 minutes sweeping the joint is closed, the combustion-tube is connected to a nitrometer, the levelling bulb of which is lowered. The sample-heater is drawn over the combustion tube and the motor is switched on. The next sample is now weighed out. When microbubbles are obtained in the nitrometer, which is the case after 40–45 minutes, the sample-heater is pushed back from the tube, joint AK is opened, stopcock BF is turned so that the carbon dioxide passes in reverse, the levelling bulb of the nitrometer is raised, boat and capsule are taken out and the next capsule and sample are introduced. After sweeping, AK is closed, the combustion tube is connected with the second nitrometer, and the next combustion is carried out. When volatile compounds are to be analyzed a quartz capillary is used or the combustion tube is cooled with dry ice or both. A tube filling can be used for about 50 analyses of 10–20 mg samples. The refilling of the tube is very easy and can be done without turning off the furnaces. The combustion tube is drawn out quickly from the apparatus in order to avoid overheating of the hopcalite. The nickel oxide filling is taken out and new nickel oxide is poured in and fixed with a small layer of asbestos. The tube is then quickly inserted again and is immediately ready for continued work.

Results obtained according to this procedure are shown in Table I. The method is also in use in the present authors laboratory on the micro scale, and work to adapt it for ultramicro-determination is going on. Trace determinations of nitrogen in mineral oil with samples of 100 mg have been carried out. A paper dealing with these adaptations of the method will be published later.

The author is much indebted to *E. Stenhagen* for his interest in the work, to *L. Nirk*, *A. Renman* and *B. Sepp* for technical assistance, to *A. Edenström*, Uppsala, *E. Svanbeck*, Nicroma, Stockholm, and *N. Rosenlind*, Norstedt & Söner, Stockholm, for assistance with constructional designs, to *R. Djurfeldt*, Bofors, for some useful suggestions and to *E. Sepp* for drawing the figures. The work was made possible by a grant from the Swedish Technical Research Council. The apparatus is available from Messrs. Norstedt and Söner, Stockholm.

Summary.

Reactions in the *Dumas* nitrogen determination method have been investigated. The presence of free oxygen in the combustion tube was found to cause retention of nitrogen oxides by the metal oxide catalyst. Nickel oxide was found superior to copper oxide because it allows the use of higher temperatures and does not liberate oxygen on heating, thus eliminating the retention error.

A new method is described which uses automatic combustion and makes use of the reaction $C + CO_2 = 2\,CO$ at 1050° C and combustion with nickel oxide at 1000° C. The standard deviation σ of 20 analyses on known compounds carried out in direct succession was 0.045%.

Zusammenfassung.

Einige bei der Stickstoffbestimmung nach *Dumas* ablaufende Reaktionen wurden untersucht. Es wurde gefunden, daß die Gegenwart von freiem Sauerstoff im Verbrennungsrohr zu einer chemischen Bindung von Stickstoffoxyden an den Metalloxydkatalysator führt. Nickeloxyd eignet sich besser als Kupferoxyd, da es die Anwendung höherer Temperaturen ermöglicht und beim Erhitzen keinen Sauerstoff abgibt, so daß der obenerwähnte Fehler vermieden wird.

Eine neue Methode wird beschrieben, nach der die Substanz durch Anwendung der Reaktion $C + CO_2 = 2\,CO$ bei 1050° C vergast und dann über Nickeloxyd bei 1000° C verbrannt wird. Die mittlere Abweichung bei 20 direkt nacheinander ausgeführten Analysen betrug 0,045%.

Résumé.

On a examiné les réactions qui se produisent dans le dosage de l'azote par le procédé *Dumas*. On a trouvé que la présence d'oxygène libre dans le tube à combustion produisait une rétention des oxydes de l'azote par l'oxyde métallique catalyseur. L'oxyde de nickel a été trouvé supérieur à celui de cuivre car il permet l'emploi de plus hautes températures et ne libère pas d'oxygène par chauffage, ce qui élimine l'erreur de rétention.

On décrit une nouvelle méthode qui utilise la combustion automatique et fait usage de la réaction $C + CO_2 = 2\,CO$ à 1050° C et de la combustion avec l'oxyde de nickel à 1000° C. L'erreur standard σ sur 20 analyses de composition connue effectuées à la suite a été de 0,045%.

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Chromatography of Organo-Metallic Complexes.

Part II.

By

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(Received August 11, 1952.)

In a previous paper[1] the authors described the separation of certain pairs of inorganic cations by chromatography of the complexes formed with sodium diethyldithiocarbamate. The solvent used was chloroform or benzene, and the chromatographic column consisted of active alumina (Peter Spence, Type H, reactivated for 3 hours at 250°–300° C).

Although satisfactory qualitative and quantitative separations were achieved with the pairs copper-cobalt, copper-ferric iron, cobalt-nickel and cobalt-ferric iron, only qualitative separations were obtained for the pairs nickel-ferric iron and nickel-copper, the two zones overlapping to some extent, so that quantitative recovery was not possible. Further investigation of the accuracy with which the minor constituent in binary mixtures of these cations may be estimated has been carried out. In the course of this investigation it was found that if the alumina was not reactivated as described in the previous paper, but was used as supplied, the ions of the nickel-ferric iron pair were less strongly adsorbed. In consequence, the two resolved bands of the separating ions moved more rapidly down the column, and separated completely into two coloured zones with a clear alumina band between them. Use of this non-reactivated alumina did not, however, give a complete separation in the copper-nickel system.

Using the method described in the previous paper (conversion of the diethyldithiocarbamate complexes to other forms and colorimetric estimation) determination of the minor constituent in all the possible binary combinations of the four cations so far investigated gave the results shown in Table I.

Table I.

Major ion	Approximate amount present, γ	Minor ion	Amount present, γ	Average amount found, γ	Percent error
Cobalt	15	Copper	3.18	3.06	4
Copper	16	Cobalt	0.73	0.72	1
Ferric	14	Copper	3.18	3.08	3
Copper	16	Ferric	0.93	0.84	10
Nickel	18	Copper	3.18	3.40	7
Copper	16	Nickel	0.90	0.51	43
Nickel	18	Cobalt	0.73	0.72	1
Cobalt	15	Nickel	0.90	0.76	15
Ferric	14	Cobalt	0.73	0.72	1
Cobalt	15	Ferric	0.93	0.83	11
Nickel	18	Ferric	0.93	0.83	11
Ferric	14	Nickel	0.90	0.74	18

It will be seen that in about half of these a highly satisfactory determination is possible. The large error in the copper-nickel pair arises from the incomplete separation on the column already referred to. Appreciable errors also arise in the determination of ferric iron and nickel in any of the combinations. In the case of the former this is due to the extremely strong adsorption of the ferric iron complex on the alumina column and the resulting difficulty or impossibility of achieving complete elution of the complex from the column after separation. The error found in the estimation of nickel probably arises from the rather faint colour of the complex on the column (yellowish-green) and the tendency to assume complete elution before this has, in fact, been achieved. The much smaller, but still significant, error in the determination of copper has already been mentioned in the previous paper and explained on the basis of residual colour of the reagent.

Summary.

The accuracy of determination of the minor constituent of binary pairs of ions, by chromatography of organo-metallic complexes and colorimetric estimation of the separated pairs, is reported. The pair nickel-ferric iron, not separated by the technique previously used, may be separated using a less highly activated alumina. Appreciable errors occur in the determination of ferric iron and of nickel, and the pair copper-nickel still remains unresolved.

Zusammenfassung.

Es wird über die Genauigkeit der Bestimmung des mengenmäßig geringeren Anteiles von binären Ionengemischen durch chromatographische

Trennung ihrer Organometallkomplexe und nachfolgende Kolorimetrierung berichtet. Das nach einem früher mitgeteilten Verfahren nicht zu trennende Ionenpaar Nickel-Eisen(III) kann zwar bei Verwendung weniger stark aktivierten Aluminiumoxyds getrennt werden, aber bei der Bestimmung des dreiwertigen Eisens und des Nickels treten merkliche Fehler auf. Das Ionenpaar Kupfer-Nickel ist nach wie vor eine ungelöste Aufgabe.

Résumé.

On fournit la précision de la détermination du constituant mineur de paires d'ions par chromatographie de complexes organo-métalliques et dosage colorimétrique des couples ainsi séparés. Le couple nickel-fer ferrique, non séparé par la technique précédemment utilisée peut être résolu en prenant une alumine moins fortement activée. Des erreurs appréciables se présentent dans le dosage du fer ferrique et du nickel et le couple cuivre-nickel n'est pas encore résolu.

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Chemical Analysis on the Microgram Scale. I.
Apparatus and Techniques.

By

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With 15 figures.

(Received August 11,. 1952.)

The apparatus and techniques which may be applied to the qualitative and semi-quantitative analysis of samples of one microgram or less have been described in great detail by *Benedetti-Pichler*[1], who seems to have been the first worker to undertake a systematic study of operations on this scale. The work which he describes includes separation of constituent ions, the identification of ions by the performance of suitable confirmatory tests, and the semi-quantitative estimation of the ions by the approximate measurement of the volumes of precipitates produced. The group of ions most fully studied comprises a portion of the sulphuretted hydrogen precipitate obtained in the classical methods of schematic analysis. Among the apparatus used by him, the micromanipulator, the "injection apparatus" for control of liquids in the pipettes, the micropipettes and the moist chamber were similar to those used by *Chambers*[2] in the examination of single living cells. Chemical operations were carried out in tiny centrifuge cones of approximately 0.5 microlitre (λ) capacity. Small amounts of reagents and wash liquids were held ready for use in reagent containers which, together with the cones, were attached by a thin layer of vaseline to a glass carrier placed inside the moist chamber. The operations were observed through a low-power microscope. The rotating mechanical stage of the microscope, together with the micromanipulator, provided means of adjusting the relation of vessels and pipettes.

After a considerable interval of time, brief descriptions of other investigations in the microgram range have been published, in which

certain modifications of *Benedetti-Pichler*'s apparatus are described[2]. The most notable of these modifications is probably the replacement of *Benedetti-Pichler*'s "injection apparatus" by a hypodermic syringe which permits simpler control of liquids in the pipettes. Little detail is given in these publication of the actual mode of operation.

In the meantime, the present authors had commenced an investigation of microgram methods, with the aim of extending *Benedetti-Pichler*'s work to other elements, and particularly with the intention of carrying out gravimetric operations using the simplified quartz microgram balance described elsewhere[3]. As a result of these investigations, considerable modifications or alterations were made in both the apparatus and the techniques described by *Benedetti-Pichler* and his co-workers. The present paper is intended to describe the modified apparatus and techniques, as they may be applied both to qualitative and to semi-quantitative analysis. Since it would be difficult to describe the modifications by themselves, without reference in considerable detail to the basic apparatus and procedures, a fairly full description is given although this will involve in some instances, particularly in the descriptions of techniques, material already described by *Benedetti-Pichler*. Anyone proposing to undertake microgram analysis is recommended to study that work thoroughly in the first instance, when the points where overlap occurs and where modifications have been introduced will then be quite apparent. At the same time the present authors wish to make it quite clear that they are fully conscious of the debt they owe to *Benedetti-Pichler*'s original investigations.

Apparatus.

Microscope: A petrographic microscope provided with a micrometer eyepiece $\times 6$ in combination with a 32 mm objective provides a convenient magnification of approximately $\times 40$. The eyepiece micrometer is calibrated for use with the 32 mm objective, the draw-tube being maintained fixed. The microscope should be fitted with a rotating mechanical stage.

Manipulator: In early work a simple manipulator providing motion in three directions at right angles, and constructed from three rack and pinion movements from discarded microscopes, was used (Fig. 1). The principal disadvantage of this was its relative instability to vibration, any vibration of the pipette tip being, of course, considerably magnified in observations under the microscope. This instrument, which was very similar in principle to the type used by *Benedetti-Pichler*, provided the experience which enabled an instrument specifically designed for this type of work to be constructed. The new manipulator, which possesses three precision motions in directions at right angles, a rapid motion which

enables the pipettes to be withdrawn quickly from the field of the microscope, and a rotation which enables the angle of inclination of the pipette tip to the cone to be altered, is illustrated in operation in Fig. 2, and its construction has been described in detail elsewhere[4].

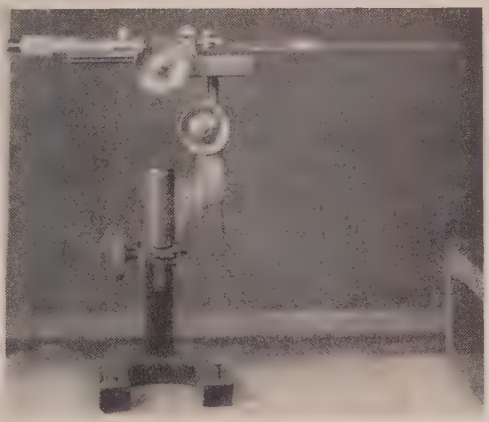

Fig. 1. Original type of micromanipulator.

Base: The manipulator can be screwed permanently to a wooden baseboard. The microscope is placed on a wooden platform of suitable height, which is also attached to the baseboard. The microscope may be positioned by wooden blocks fastened to the platform, but it need not be permanently fixed. No difficulty should be experienced in replacing it immediately in the working position, since the mechanical motions of the manipulator and microscope-stage provide sufficient flexibility.

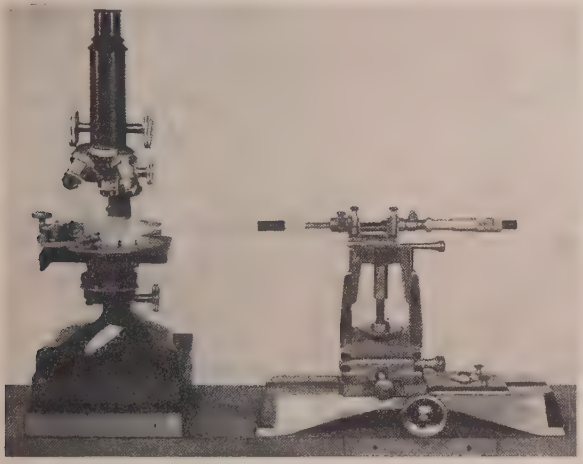

Fig. 2. Complete set-up for microgram analysis, with new type of micromanipulator.

Illumination: With the apparatus to be described normal daylight illumination is sufficient for most purposes. In order to observe some

of the confirmatory tests it is sometimes desirable to illuminate the plateau of the condenser rod (p. 148). For this purpose a beam of light is thrown horizontally from a pencil-type electric hand torch through the base of the micro-condenser, to illuminate the plateau by internal reflection.

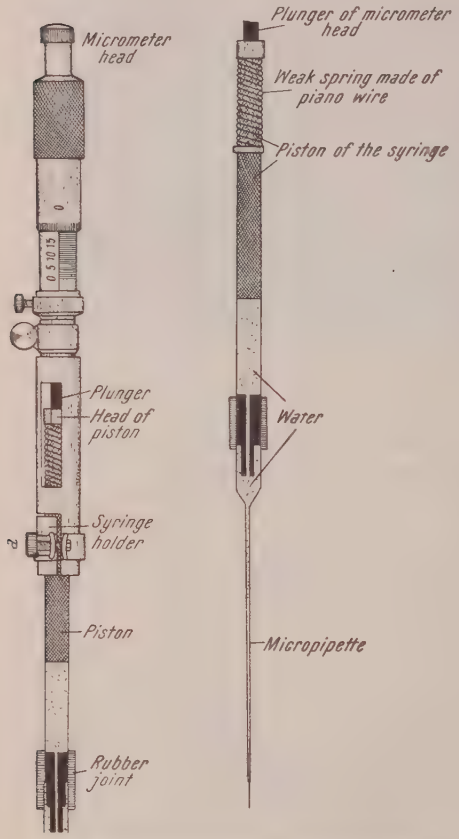

Fig. 3. Micrometer syringe and micropipette.

Micrometer syringe: This is an Agla micrometer syringe*, and is illustrated in Fig. 3. The plunger of the micrometer head presses directly on the piston of the syringe. The syringe is held in a metal framework. Around the stem of the piston, and held between the head of the piston and the lip of the syringe-barrel there is placed a weak spring constructed from piano wire. Springloading in this fashion ensures that the piston advances or is retracted with the corresponding movement of the micrometer plunger. The syringe is attached to the micromanipulator either (old pattern) by spring clips, or by hinged screw clips which are cork lined. Moderate pressure of the clips holds the syringe firmly in position. This device for the control of liquids in the micropipettes is very much simpler than the screw control, copper tube and pipette-holder described by *Benedetti-Pichler*.

Micropipettes: A soft glass tube of approximately 8 mm outer diameter (similar to the syringe barrel), 6 mm bore and 10 cm long is heated at the centre, preferably in a narrow flame such as a bats-wing burner. It is then drawn symmetrically, outside the flame, to produce a capillary of approximately 1 mm outer diameter, the pull being maintained until the glass cools. This produces a straight capillary with part of the original tubing attached at each end (Fig. 4a). Cutting the stem at the proper

* Messrs. Burroughs Wellcome.

point will produce two similar pieces of the original tubing (the "stem") with 15 cm of capillary attached (Fig. 4b). Excess of the stem is cut off approximately 2 cm from the capillary, preferably using a "hot point" since vibration may break the capillary. The 1-mm capillary (the "shank") is then drawn out by hand at the end furthest from the original tubing as follows:—the capillary is held horizontally, being grasped between the thumb and forefinger at the point x, approximately 6–8 cm from the stem (Fig. 4b). The thumb and index finger of the other hand grasp the capillary at the point y, 4 cm from x. The hands are steadied by resting the edges of the palms on the bench. The capillary is then brought over a microburner with a pinhead flame, so that it is heated at z, midway

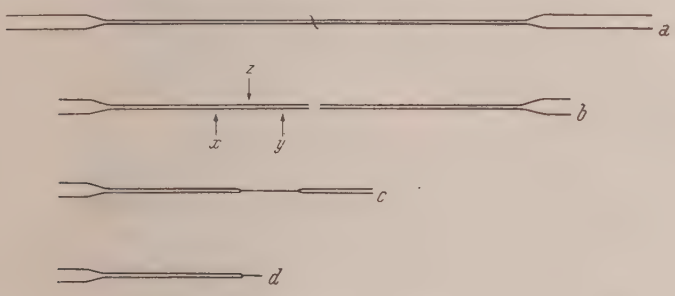

Fig. 4. Construction of micropipette.

between x and y until the glass softens. The capillary is immediately withdrawn from the flame, and a horizontal pull is applied symmetrically by rolling the edges of the palms on the bench. The amount of extension is usually 3–5 cm (Fig. 4c). Cutting at the proper point will leave 8–10 mm of fine capillary (the "shaft") attached to the 1-mm capillary (Fig. 4d). The diameter of opening which is most suitable is 20–50 μ. Trials will result in acquiring the proper technique for preparing a suitable micropipette.

To eliminate surface tension effects which may otherwise cause considerable difficulty in operation, resulting in poor control of liquid flow, the micropipette tip is then treated in one of the following ways:—

(i) The tip of the pipette is dipped in Teddol*. Suction is applied through the stem till the liquid fills the shaft, the taper and a short length of the shank**. After a few minutes the liquid is expelled. This causes the surface in contact with the liquid to become water repellent. The remainder of the inner surface of the shank also becomes water repellent

* Mixed methyl chloro silanes. Messrs. British Thompson Houston, Ltd.

** Care must be taken not to inhale the vapours of the liquid. Suction applied by the mouth is not recommended.

to some extent through contact with the vapours. Surface tension effects are completely eliminated. It is convenient to transfer a bulk supply of Teddol to small ampoules made from 2-mm capillary tubing, only opening one of these when a stock of pipettes is ready for treatment. In this way waste of the liquid is avoided.

(ii) The tip of the pipette is treated with Akard*. One part of Akard is diluted with 3–4 parts of absolute alcohol. The shaft of a micropipette to be treated with the solution is cut so that it is 5–7 mm longer than the normal value already described. The tip is just dipped in the diluted liquid, and suction is applied until the liquid fills the shaft, the taper and a few mm of the shank. The liquid is then expelled. The pipette is left to air dry for a short time, and it is then "stoved" by heating for two hours at 140–150° C. The portion of the tip which was dipped in the liquid is then cut off, so that the coating only remains on the inside wall. This is necessary since some precipitates have a tendency to adhere to the coated surface.

Of these two treatments, that with Teddol has been found most serviceable. In preliminary work the authors used the technique described by *Wigglesworth*[5] of coating with paraffin wax. This is quite satisfactory for much qualitative work, but was discarded in favour of one of the other treatments described here when quantitative work was undertaken, since it was found that occasionally the wax coating or a portion of it became detached and could thus contaminate the precipitate.

The micropipettes described here differ from those used by *Benedetti-Pichler* in a number of features. They have a stem suitable for direct attachment to the glass barrel of the hypodermic syringe through a simple rubber joint instead of through the rather elaborate pipette-holder. The water-repelling coating results in highly efficient control over the volumes of solution taken up or delivered. It is therefore a simple matter to construct a satisfactory hand-drawn micropipette, avoiding the necessity for using elaborate mechanical devices[6] for drawing pipette-tips.

Assembly of the pipette-syringe combination: The two screws *a* of the syringe holder (Fig. 3) are released, and the clip which they control is detached. The glass syringe and spring-loaded piston are removed from the holder. The syringe is filled with distilled water which has previously been boiled to drive out dissolved gases, and which is introduced while still lukewarm. Filling is carried out by holding the syringe nearly vertical, tip downwards and dipping in the water, and allowing the plunger to travel gradually backwards under pressure from the spring to within 1 cm of its complete travel. Care is taken that no air bubbles are taken

* A solution of partially polymerised phenol-formaldehyde resin. Messrs. I. C. I., Ltd.

up. In this way enough water is taken up to ensure that the stem and part of the shaft of the micropipette will be filled with water when the plunger of the syringe is advanced to the half-way point.

After filling, the syringe is reversed so that the tip points upwards. A micropipette is fitted with a rubber joint, so attached that half its length covers the stem of the pipette. The rubber is moistened to facilitate insertion of the syringe barrel, which is then pushed into place. The piston of the syringe is then advanced very gradually through the barrel until the pipette stem and part of the shank are filled with water, and no air bubbles are trapped. The syringe and pipette are than placed approximately in position in the clamp attaching them to the micrometer, taking care to prevent the piston from sliding back and trapping air. The plunger of the micrometer is brought up into position against the head of the piston, and is adjusted to bring the meniscus of the water about one-third way along the shank from the stem. If necessary, the position of the syringe in the clamp receives a final adjustment. The clip is replaced, and the screws, *a*, are tightened. The meniscus of the water is again adjusted, if necessary, using the micrometer, and the pipette is now ready for use.

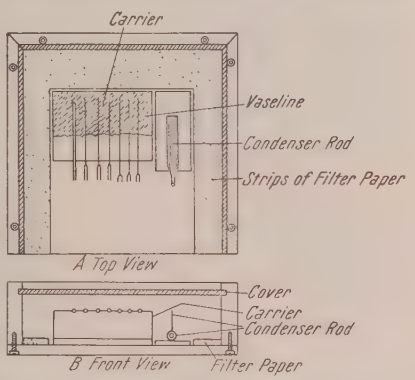

Fig. 5. Moist chamber.

Loss of water in the barrel of the syringe occurs through evaporation, and after continued use. After a micropipette has been in use for some time it is therefore necessary to supplement the water. For this purpose it is not necessary to detach the pipette from the syringe. The piston of the syringe is advanced until a small droplet of water forms at the tip of the pipette. The tip is then immersed in warm, freshly boiled distilled water, and the piston is retracted very gradually till sufficient water has been taken up. Any excess of water taken up can be removed in the same way through the tip.

Moist chamber: The bottom, sides and back of the moist chamber (Fig. 5) are made of Perspex. The cover, which is of glass, fits into horizontal grooves in the sides and back of the cell, sliding freely, but not fitting too loosely. It is thus readily removed. In the centre of the back panel a small removable plug of Perspex closes a 1-cm diameter hole which permits of adjustments being made from the back if necessary. This, however, is rarely used.

To maintain a moist atmosphere in the cell, strips of filter paper several layers thick are laid on the bottom of the cell as shown, and these are kept moistened with water. The cell is held and operated by the mechanical stage of the microscope. The dimensions of the cell may vary within considerable limits, depending on the size which the mechanical stage of the microscope is able to accommodate. It is, however, desirable that with this limit in mind the floor area should be as large as possible. An internal vertical depth of about 2 cm is convenient.

This cell is easier to construct than that described by *Benedetti-Pichler* and his co-workers, and has the advantage of light weight and complete illumination, since brass is not used.

Carrier: Two types of carrier may be used. That illustrated in Fig. 6 is built up from pieces of microscope slide cemented together and stuck on a rather larger glass base. A handle may be attached to move the carrier in and out if desired.

Fig. 6. Glass carrier.

Fig. 7. Plastic carrier.

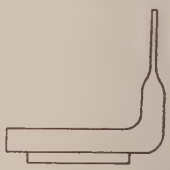

Fig. 8. Condenser rod.

Alternatively, a carrier of the same general shape may be constructed from a small block of Perspex cemented on to a Perspex sheet base (Fig. 7). A groove is then cut round the platform as shown, which takes a light rubber band under which the handles of cones and containers are inserted. The groove must be cut deeply on the base to avoid affecting the sliding of the carrier in the cell. This type of block obviates the use of vaseline for sticking the handles of vessels to the surface of the platform. It is essential in quantitative work where the vessels have subsequently to be weighed.

Condenser Rod: The condenser rod shown in Fig. 8 is constructed from glass similarly to that of *Benedetti-Pichler*. It is more satisfactory to coat the vertical thread with Teddol than with paraffin wax. The thread is then cut at the appropriate point to provide the platform. Efficient illumination is provided by casting a beam of light horizontally through the broad end as shown by the arrow, using a pencil-type electric hand torch.

Reagent containers: There are prepared from glass capillaries approximately of 0.5 mm uniform bore, which have been drawn out from clean quill tubing and have been cut into pieces approximately 15 cm long. The bore is measured at both ends of each capillary, and the maximum deviation from the arithmetical mean should not be more than 5 per cent.

To measure the bore of the capillary, the capillary is placed in a piece of narrow quill tubing of suitable length, fixed vertically to a movable support. The condenser and mirror of the microscope are removed, the end of the capillary is brought under the objective of the microscope, and is sharply focussed, being then measured by the eyepiece micrometer.

Using a microburner having a jet opening of about 2 mm and a flame about 0.7–1 mm long, the capillary is fused to form an elongated bead at a point approximately 1–2 cm from the end (Fig. 9a). This bead is then drawn out, outside the flame, to a thin rod approximately 4 cm long (Fig. 9b). This operation is repeated at 2 cm intervals until a "chain-like" structure consisting of parts of the original capillary 2 cm long, joined by solid threads, results, as shown in Fig. 9c. Cutting at the proper

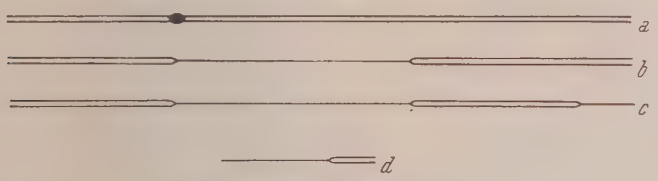

Fig. 9. Construction of reagent containers.

points gives containers each consisting of 5–8 mm of the original capillary attached to a thread about 2 cm long. These are stored in a screw-cap phial.

Cutting of capillaries can conveniently be achieved using a thin-edged carborundum plate. The most satisfactory cutter, however, is a thin slip of tungsten carbide machined to provide as sharp an edge as possible. This should be reserved for capillary cutting, and should not be used on ordinary glass-cutting operations, as otherwise the cutting operation will require too much pressure as soon as the cutting edge becomes slightly blunted.

Containers of the pattern described may be prepared rather more rapidly than using the method described by *Benedetti-Pichler*.

The length of container which holds one millimicrolitre (1 mλ) of liquid is calculated from the average diameter of the bore of the original capillary. The length of capillary which holds 1 mλ is most conveniently expressed in terms of the eyepiece micrometer divisions, and is recorded on the labelled phial.

Capillary cones: These are prepared as a "chain" exactly in the same way as the reagent containers. From the chain, blanks are obtained by cutting the middle of each glass thread, giving pieces of capillary about 2 cm long with a glass thread attached at each end, as shown in Fig. 10a. The sealed ends of these lengths of capillary are then examined carefully

under the microscope. The tip of a capillary cone must be completely closed, and the taper should be rather blunt, as shown in Fig. 10c. If the end is satisfactory, cutting at the proper point produces a cone about 2 mm in length with a handle about 2 cm long, as shown in Fig. 10b. Approximately one-half of the cone should be given to the taper. Capillaries whose ends, on examination, prove to be unsuitable for use as cones (as, for example, the types illustrated in Fig. 10d, e) may be reserved for cutting to form reagent containers.

The use of a micro-flame, instead of the edge of the Bunsen flame, as described by *Benedetti-Pichler* and his co-workers, permits better observation and control of the process, and appears to give wider-angled cones. Such cones permit convenient agitation of a precipitate by the pipette tip, which can reach effectively to the point of the taper. Furthermore, the technique of preparation is less time-consuming.

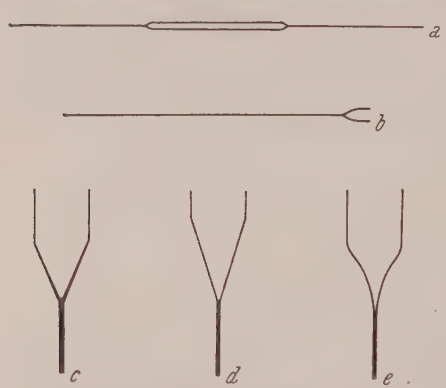

Fig. 10. Construction of capillary cones.

Measuring capillaries: These are thin-walled capillaries approximately 0.2–0.3 mm bore, uniform, and 2–3 cm long. They are drawn from thinwalled tubing 3–5 mm in bore. The bore of each capillary is measured at each end in order to ensure uniformity, and one end is then sealed in a micro-flame.

The length of each capillary which holds one millimicrolitre is calculated in the same way as for the reagents containers, and is recorded. Each measuring capillary is stored separately in a piece of wide capillary sealed at each end and labelled with the content.

Measuring capillaries may be treated with Teddol before sealing to make the inner surface water repellent. Indeed it is possible, by using a dilute solution of Teddol rather than the liquid itself, to achieve an interior surface such that the meniscus is flat. With stronger treatment the meniscus is, of course, rounded in the reverse sense to that with a plain glass surface.

Storage blocks for cones, etc.: Small Perspex or other plastic blocks are machined to give a slight circular projection on the upper surface. The face of this projection is then drilled with small holes which will take the handles of cones or reagent containers. The centre of the block is drilled with a hole of rather larger diameter, and this is filled with cotton-wool which is kept moist. The whole is covered with the lid of

a Petri dish or with a crystallising dish which just fits over the circular platform, providing a clean storage container for vessels which are in the course of use for chemical operations (Fig. 11).

Heating block: This is a cylindrical block of aluminium or duralumin with a central well for the thermometer and holes of various diameters for the insertion of capillaries (Fig. 12). It is heated by a small microburner having a narrow orifice (the base of a Bunsen burner from which the tube has been removed is satisfactory). The flame is adjusted to give the required temperature. A block of this type offers the simplest means for the maintenance of controlled temperature.

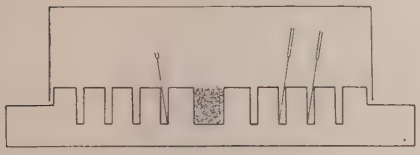

Fig. 11. Storage block for working vessels.

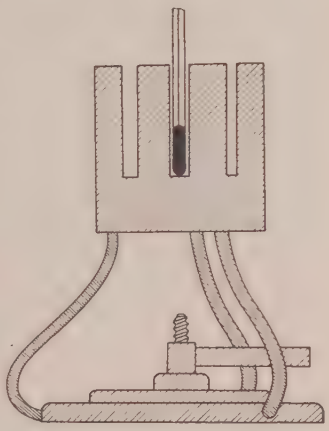

Fig. 12. Heating block.

Glass rod for "thread" tests: This is a straight piece of glass rod approximately 0.5 cm in diameter and 2.5–3 cm long, or a bent glass thread, fixed on the carrier. To this a thin cotton thread can be attached as shown in Fig. 13. On this thread certain confirmatory tests can be performed.

Centrifuge. A hand centrifuge may be used as described by *Benedetti-Pichler*, taking precautions to ensure as uniform centrifuging as possible when performing semi-quantitative determination. Alternatively a motor-driven microcentrifuge is convenient.

Fig. 13. Holders for thread tests.

Projection apparatus: If any amount of ultramicro work is to be carried out, it will prove a great convenience to have the microscope fitted with a projection apparatus giving an image on a vertical screen facing the operator, thus avoiding eyestrain and enabling the operator to work in a more comfortable position.

Techniques.

The basic operations are described in some detail though this may once more involve some repetition of descriptions already provided by *Benedetti-Pichler*. It would be both difficult and, indeed, confusing,

to restrict this section to isolated descriptions of the ways in which the earlier techniques have been modified.

It will be assumed that the apparatus has been prepared and assembled as already described. The micropipette should be properly attached to the glass syringe and in position on the micromanipulator. The meniscus of the water in the shank of the pipette should be approximately one-third of the way from stem to tip. The eyepiece micrometer should have been calibrated in conjunction with the eyepiece and objective in use, and the tube length fixed. The moist chamber is placed on the mechanical stage of the microscope with its open side facing the micromanipulator and micropipette, and is fitted with moist filter paper, a condenser rod, reagent containers, capillary cones, measuring capillaries, and a glass rod for thread tests.

Charging reagent containers with solution: For this it is convenient to use capillary pipettes having a shaft of comparatively wide bore

Fig. 14. Filling reagent container.

(approximately 0.2 mm) and from 0.7–1 cm long, instead of the normal shaft of about 0.05 mm bore and 2 cm length. The pipette is first charged with solution by inserting the tip into a drop of the required reagent, which may be held either on the end of a glass rod or in a wide capillary. When sufficient liquid has been taken up, the tip is withdrawn, and is inserted well into the reagent container. Liquid is gradually expelled, and at the same time the tip is slowly retracted, so that the solution flows freely, and fills a length of the container (Fig. 14) without bubbles.

Measuring a sample.

1. *Withdrawing a measured volume from the pipette:* By means of the mechanical stage, and using transmitted light, the reagent container holding the sample is brought within the field of view, and its position is then adjusted so as to occupy approximately one-half of the field. The focus is adjusted so that a sharp image of the mid plane of the capillary is obtained. In this way the walls of the capillary appear as sharp straight lines.

By operating the rapid lateral control of the micromanipulator, and using direct observation, the micropipette is introduced into the moist cell and its tip is brought close to the opening of the container by appropriate use of the other controls. Observing through the microscope while operating the vertical motion of the micromanipulator, the tip of the micropipette is brought sharply into focus. This ensures that it is now

in the same horizontal plane as the axis of the reagent container. Using the rotating stage of the microscope and the tilting control of the manipulator, the container and the pipette tip are adjusted parallel to each other and to the lateral axis of the complete assembly. Finally the transverse motion of the manipulator is used to bring the pipette into line with the axis of the container. Using the fine lateral control, the tip of the pipette is advanced to lie just within the opening of the reagent container.

The eyepiece is rotated, if necessary, to bring the micrometer scale parallel to the image of the reagent container. The position of the reagent container is adjusted by means of the mechanical stage so that the meniscus of the liquid in the container coincides with a convenient scale division in the eyepiece. The pipette is cautiously advanced by the fine lateral control of the manipulator until the tip is immersed slightly further than the length of liquid which it is proposed to remove. Suction is applied cautiously by turning the micrometer head. The meniscus recedes in the reagent container. When slightly more than the required amount of liquid has entered the pipette, the micrometer head is adjusted to reach equilibrium and to maintain the meniscus of the liquid perfectly steady in the container at the desired scale division. When this state of equilibrium is achieved, the fine lateral control of the manipulator is used to withdraw the pipette.

2. *Accurate measurement of the volume:* The pipette tip is withdrawn a short distance from the container by the fine lateral control. It should be visible a few scale divisions from the container mouth, so that part of the shaft can still be seen within the field of view. Using the mechanical stage, and under direct observation, a measuring capillary is brought into the position previously occupied by the reagent container. Viewing through the microscope, adjustments are carried out as before to bring the pipette and the measuring capillary into correct alignment. The final adjustment is carried out with the tip of the pipette just inside the mouth of the measuring capillary, and the pipette is then advanced by a distance which is assessed to correspond to the amount of liquid to be measured.

On cautiously beginning to expel the liquid, a droplet forms at the tip of the pipette, and this grows until it fills the bore of the measuring capillary. Two menisci form which continue to move apart. At this point the pipette is gradually retracted so that it follows the outer meniscus without being removed from the liquid. Pressure is finally adjusted by the micrometer to produce stable menisci the calculated distance apart for the volume required.

The pipette is then withdrawn from the capillary using the fine control, and is withdrawn completely from the field of the microscope using the

coarse lateral control. Excess of the solution is completely expelled and the droplet is received on a strip of filter paper.

3. *Withdrawing the measured sample from the capillary:* In the same way as before the pipette tip is brought forward and adjusted to come just within the outer meniscus of the liquid. Very gradual suction is applied by the micrometer head. At the same time the meniscus is maintained in the same position relative to the pipette tip by advancing the capillary using the mechanical stage. When the liquid has been taken up as completely as possible, pressure on the pipette is adjusted so as to form a tiny droplet at the pipette tip. This is touched to any drainings still remaining on the wall of the capillary, by using either the tilt control or the rotating stage, so that the shaft of the pipette makes a slight angle with the capillary wall. Application of slight suction, perhaps accompanied by gradual retraction of the capillary, will take up the drainage. Great care should be taken throughout, particularly at this stage, to secure an uninterrupted flow of liquid from the capillary to the pipette, so that air bubbles are not trapped. Otherwise these will be expelled during subsequent delivery, and may cause spattering of the liquid.

The pipette is then retracted so that its tip remains visible near the edge of the field of the microscope.

4. *Transfer of the measured sample to the capillary cone:* The selected cone is brought into the field and the microscope is focussed on its axial plane. For this purpose observation may be directed towards the point of the taper. Using the mechanical stage the position of the cone is adjusted so that the vessel occupies approximately one-third of the field. The pipette is adjusted and advanced so that its tip comes close to the opening of the cone, and it is brought into sharp focus using the vertical motion of the manipulator. The cone is now advanced so that the pipette tip touches the wall of the cone lightly near the point of the taper. Liquid is ejected slowly, and simultaneously the cone is retracted so as to maintain the tip of the pipette just inside the meniscus.

When the meniscus in the pipette approaches the opening, flow of liquid is stopped. The cone is withdrawn slightly till the tip of the pipette is just removed from the liquid. The stage of the microscope or the tilt of the manipulator is adjusted to allow the opening of the pipette to touch the wall of the cone close to the meniscus, while the pipette makes an angle of 30–40° with the axis of the cone. The remainder of the solution is expelled very gently. The cone is retracted, and the micropipette is removed from the moist cell for cleaning.

The micro cone now contains an accurately measured volume of solution.

Cleaning the micropipette: Washing by distilled water is carried out by advancing the piston of the syringe until water moves up the pipette

from the syringe and forms a droplet at the tip of the pipette. This droplet is removed by a strip of thin filter paper. Another droplet is formed in the same way and is removed. The piston is then retracted until the water meniscus returns to its original position.

If other wash liquids have to be used, small volumes of these are taken up in the pipette by slight suction so as to fill the shaft and taper, and the liquid is then expelled on to a strip of filter paper. The drop of wash liquid may be held on a microscope slide, in a wide capillary, or as a hanging drop on a glass rod. The shaft and tip of the pipette are so flexible that no serious danger of breakage occurs if the tip touches the slide or other holder.

Measuring a reagent: A reagent is brought into the pipette in the same way as already described for the withdrawal of a measured volume from a reagent container. This gives an approximate amount of reagent, which is sufficient for most purposes, and it is usually not necessary to carry out an accurate measurement in a measuring capillary.

Addition of reagent to the solution in the cone: The tip of the pipette is set so that it is visible near the edge of the field, while the cone is placed so as to occupy about one-third of the field. Using the methods already described for adjustment, the pipette is brought close to the opening of the cone, and is sharply focussed by means of the vertical adjustment of the manipulator. The cone is then advanced so that the tip of the pipette is close to the surface of the liquid already in the cone. By slight pressure the liquid in the pipette is adjusted so as to fill the tip completely without forming a droplet. The cone is then further advanced to bring the tip in contact with the meniscus, upon which delivery of the reagent commences. By continued slight pressure nearly all the reagent is expelled, the cone being gradually retracted as delivery takes place. Great care must be taken that the shaft of the pipette does not touch the wall of the cone, as liquid and precipitate may then spread along the wall beyond the normal meniscus level.

When almost all the reagent has been expelled the cone is retracted slightly to bring the pipette tip out of the liquid. The remainder of the reagent may then either be delivered on the wall of the tilted cone close to the meniscus, or may be discarded.

Stirring the contents of the cone: This can be done by moving the shaft of the pipette a few times backwards and forwards through the solution. The opening of the pipette is sufficiently sealed by the last traces of reagent remaining in the tip.

Heating the contents of the capillary cone: A little water is allowed to enter at one end of a capillary about 5 cm in length and about 1 mm bore. The end is then sealed in a micro flame. The tube is allowed to cool and the water is collected at the sealed end by shaking or centrifuging.

The capillary cone is removed from the moist chamber. If it has been attached by vaseline to the platform it is laid on a sheet of filter paper and the handle is cleaned by rolling on the filter paper. The cone is then introduced, handle first, into the capillary, and is allowed to slide down to the sealed end. If necessary, a glass thread which just slides inside the capillary may be used to push the cone down.

The open end of the capillary is then sealed, and the capillary is inserted in the appropriate hole of a heating block at the required temperature. The bore of the heating capillary is of importance. When heating for several minutes just below 100° C, wide capillaries appear to encourage partial evaporation from the cone, while narrow capillaries encourage dilution of the solution in the cone.

Because of the possibility of slight evaporation, it may be advisable to centrifuge the contents of the cone by applying a few slow turns of the hand centrifuge, just before insertion in the heating block. This brings the precipitate a short distance below the meniscus, so that subsequent evaporation does not leave dried precipitate sticking to the walls where it might escape detection or estimation. This step, however, is not always essential.

After cooling, the upper end and then the lower end of the heating capillary are cut off. Using a glass thread inserted from the lower end, the cone is pushed out through the upper end and is caught with forceps.

Centrifuging the contents of the cone: The cone is introduced into a capillary approximately 4 cm long and about 1 mm in bore which is held almost horizontally. Still maintaining this in the horizontal position, the centrifuging capillary is inserted into an ordinary centrifuge cone so that the handle of the micro cone is pointing towards the tip of the large cone, which is then placed in position in the centrifuge.

After centrifuging, the outer cone is again held horizontally, and the centrifuging capillary and micro cone are withdrawn. The cone may be withdrawn at the lower end if the tip of the handle protrudes slightly, or it may be pushed to the top by means of a glass thread.

Cones may be centrifuged directly in a heating capillary after heating, the ends of the capillary first being cut off and any residue of the drop of water being taken up by a small strip of filter paper.

Observing a precipitate in the cone: The colour of a precipitate is best observed under reflected light.

Separation of solution and precipitate: The micropipette and cone are adjusted so as to let the tip of the pipette enter the solution in the cone and reach a short distance below the meniscus. Suction is then cautiously applied, maintaining the tip just below the meniscus until nearly all the liquid has been removed. The last traces of the liquid are removed very cautiously to avoid disturbing the precipitate or taking any

of it up in the pipette. The cone is then retracted, the pipette is withdrawn, and the liquid contained in it is either transferred to another cone if it is to be treated further, or is discarded.

Washing the precipitate: An approximately measured volume of wash liquid is taken into the pipette from a container, and is slowly delivered on the wall of the cone close to the precipitate, in such a way that the precipitate is covered with wash liquid without being disturbed. The cone is retracted as the amount of liquid in it increases, so that, as usual, the tip is always just below the surface. The wash liquid is left for a short time in contact with the precipitate, and is then withdrawn by the pipette and rejected. The operation is repeated as often as desired.

Depositing solution on the plateau of the condenser rod: An approximately measured volume of test solution is taken up in the micropipette, and the tip is then moved so that it is just visible in the field. By direct observation the platform of the condenser rod is brought close to the tip of the pipette, and hence within the field of view. Under the microscope the plateau is sharply focussed at the furthermost third of the field from the pipette. The pipette is brought up to the plateau, brought into focus by the manipulator, and then raised slightly so as to bring it just out of focus. It is then advanced to occupy one-third of the plateau, and is lowered so that the opening just comes into contact with the plateau and is therefore almost in sharp focus.

Liquid is expelled slowly on to the surface of the condenser rod. The pressure is reduced near the end of delivery, and when the meniscus almost reaches the tip of the pipette this is slightly raised, and the pipette is removed from the moist chamber.

Addition of reagent to the plateau and observation of results: The required volume of reagent is measured off by the pipette. Lateral illumination is applied to the condenser. The reagent is added slowly to the solution already on the plateau, care being taken to apply a slight pressure before the tip enters the solution. Liquid thus flows immediately on touching the pipette tip to the test solution, preventing deposition of precipitate inside the pipette tip. When the reagent has been delivered the pipette is withdrawn and cleaned.

Preparing the plateau for further operations: After a confirmatory test has been applied the condenser rod is taken out of the moist cell, and the precipitate is removed by dipping the plateau and part of the thread in the proper solvents, finally rinsing in the same way with distilled water.

If this treatment fails to clean the plateau completely, a new surface is prepared by cutting the thread a short distance below the original surface. This may be repeated until the thread becomes too short for further cutting.

Performing a confirmatory test on a thread: A fine piece of cotton is attached by gum or starch paste to the sides of a straight cut piece of glass rod so that the thread makes a small loop. Alternatively the thread may be attached to the two ends of a bent glass thread. These two variants are shown in Fig. 13. The thread is then impregnated with the required reagent throughout most of the loop, the reagent being applied as a hanging drop from the end of a glass rod, or from a capillary. The rod is then placed on the carrier so that the thread is horizontal. An approximately measured amount of test solution is taken up in the pipette, which is than manipulated so that its tip is almost in contact with the thread. Slight pressure is applied to form a droplet at the opening of the micropipette. The pipette is then advanced to allow the thread to break the surface of the droplet. After a little wait the whole test solution is discharged slowly on to the thread.

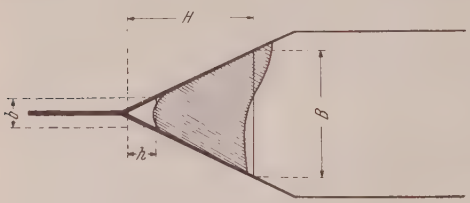

Fig. 15. Measurement of volume of precipitate.

Removal of excess of bromine or other gas from a solution: This is achieved by alternately sucking the solution into the pipette and discharging it. With bromine the colour of the solution gradually fades and it finally is completely discharged.

Estimation of the volume of a precipitate: For semi-quantitative operations, cones with straight-sided symmetrical blunt tapers must be used. Centrifuging must be carried out using a constant procedure, so that a reasonable reproducibility of results and a satisfactory comparison with standard precipitates is possible. Thus, with a hand centrifuge the crank of the instrument may be turned strongly 20 times. However, an electrically driven instrument is less liable to variation.

The precipitate is observed using transmitted light, with the microscope focussed on the point of the cone-taper. The approximate volume of the irregular truncated cone of precipitate is estimated, as described by *Benedetti-Pichler*, using the formula

$$V = 0.26\,(B^2\,H - b^2\,h)$$

where the dimensions, shown in Fig. 15, are measured by the calibrated eyepiece micrometer and converted to microns.

With wide-angle cones and fine precipitates the tip of the cone may be nearly or completely filled with precipitate, so that this may be regarded as forming a complete cone. In such a case the formula reduces to

$$V = 0.26\,B^2\,H.$$

In this formula V represents the volume of the precipitate in cubic microns. This may be more conveniently expressed in millimicrolitres, obtained as $V/10^6$.

Summary.

The apparatus and techniques which may be used to carry out qualitative and semi-quantitative analytical operations on the microgram scale are described in detail.

Zusammenfassung.

Die technische Durchführung qualitativer und halbquantitativer Analysen im Gamma-Maßstab und die hierfür geeigneten Geräte werden eingehend beschrieben.

Résumé.

On décrit en détail l'appareillage et les techniques qui peuvent être utilisés pour effectuer les opérations analytiques qualitatives et semi-quantitatives à l'échelle du microgramme.

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Aus dem Medizinisch-chemischen Institut der Universität Zagreb.

Ein einfaches Mikrocoulometer.

Von

Ivan Berkeš.

Mit 1 Abbildung.

(Eingelangt am 14. August 1952.)

Für unsere Arbeiten auf dem Gebiet der Papier-Mikroelektrophorese[1, 2] ergab sich die Notwendigkeit der Kontrolle der mittleren Stromstärke, des gesamten Stromverbrauches und allenfalls eingetretener Stromunterbrechungen während des Versuches. Diesen Zweck erfüllt vollständig ein einfaches Gas-Coulometer, das aus jeder Bürette leicht hergestellt werden kann. Man bestimmt den Büretteninhalt vom Glashahn bis zum Ende der Bürettenskala möglichst genau mit Quecksilber. Die mit dem offenen, hahnlosen Ende nach unten, also verkehrt befestigte Bürette wird mittels eines Gummistopfens mit einem kleinen Ansatzgefäß aus Glas (Abb. 1) verbunden, in dessen Wandung zwei Platinelektroden eingeschmolzen sind und das einen seitlichen Ansatz zum Anschluß einer Niveaubirne trägt. Nach oben verjüngt sich dieses Glasgefäß zu einem Rohr von 4 mm äußerem Durchmesser, das durch den Gummistopfen ein wenig in die Bürette vorragt. Der Apparat wird mit 10%iger Schwefelsäure gefüllt und die Elektroden angeschlossen. Nach Beendigung des Versuches wird auf gleiches Niveau eingestellt und das Volumen des gebildeten Knallgases gemessen, das selbstverständlich auf Normalbedingungen reduziert werden muß. Für besonders genaue Messungen ist auch die Löslichkeit des Gasgemisches in der Schwefelsäure zu berücksichtigen.

Bei Verwendung einer gewöhnlichen Makrobürette mit 0,1-ml-Teilung kann auf 0,05 ml genau abgelesen werden. Der Fehler beträgt bei 50 ml Gas also $\pm 0{,}1\%$. Bei Mikrobüretten ist für ein Gasvolumen von 5 ml mit der gleichen Fehlergröße zu rechnen. Die Zuverlässigkeit der ganzen Anordnung hängt außer von der einmaligen Ausmessung mit Quecksilber (s. oben) ausschließlich von der Genauig-

keit der Bürettenteilung ab, da Fehler bei der Messung von Druck und Temperatur praktisch überhaupt nicht ins Gewicht fallen.

Bei Ermittlung einer Stromstärke von 4 mA ist also z. B. mit einem Fehler von $\pm 0{,}004$ mA zu rechnen. Selbstverständlich vergrößert sich dieser Fehler bei kleineren Knallgasmengen und sehr kurzen Versuchszeiten. Ein Ablesefehler von 0,05 ml entspricht nach Reduktion auf Normalbedingungen weniger als 287 Milliamperesekunden. Bei nur einstündiger Versuchsdauer beträgt also die Abweichung vom tatsächlichen Wert der mittleren Stromstärke weniger als 0,08 mA.

Ohne daß die verwendete Bürette für andere Zwecke unbrauchbar gemacht würde, ist also mit Hilfe des kleinen Glasansatzgefäßes ein präzises Meßinstrument herstellbar.

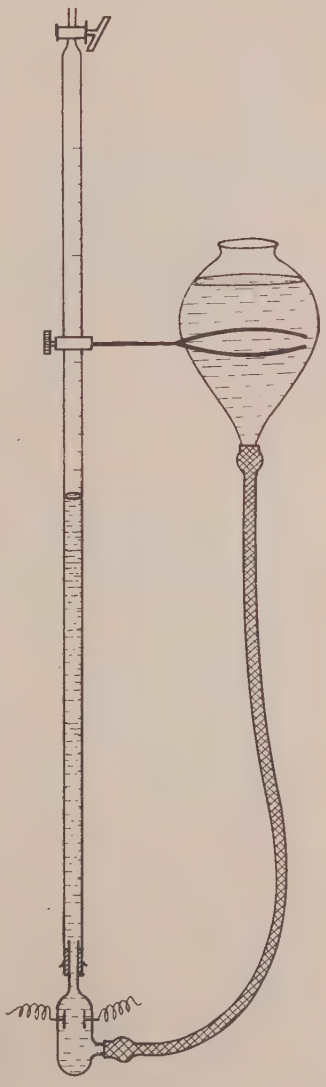

Abb. 1.

Zusammenfassung.

Es wird ein einfaches Gas-Coulometer beschrieben, das aus einer Bürette und einem Glasansatzgefäß mit zwei Platinelektroden herstellbar ist. Mit dessen Hilfe sind kleine Strommengen und Stromstärken mit hoher Genauigkeit meßbar.

Summary.

A simple gas coulometer is described, which can be constructed from a burette and a glass extension piece with two electrodes. Small amounts of current and current strengths can be measured with high accuracy by its aid.

Résumé.

On décrit un coulomètre à gaz qui est fabriqué avec une burette et un dispositif de verre avec deux électrodes de platine. On peut mesurer avec lui de petites quantités de courant et des intensités avec une grande précision.

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Zur mikromaßanalytischen Schnellbestimmung des Schwefels in organischen und anorganischen Substanzen.

(4. Mitteilung[1].)

Von

Wilhelm Zimmermann.

Mit 1 Abbildung.

(Eingelangt am 20. August 1952.)

Nach der Bekanntgabe der Methode im Jahre 1943 sind inzwischen vier weitere Veröffentlichungen, zwei eigene und zwei fremde[2, 3], erschienen, die sich mit der mikroanalytischen, volumetrischen Bestimmung des Schwefels beschäftigen. In engem Zusammenhang damit steht auch eine andere Arbeit, die den Reaktionsvorgang beim Kaliumaufschluß organischer Substanzen näher untersucht[4].

Die Tatsache daß der Bedarf an Schwefelbestimmungen immer sehr groß ist, schuf die Möglichkeit der Weiterentwicklung der Methode, deren Ergebnisse im folgenden mitgeteilt werden sollen; um Mißverständnisse von vornherein auszuschließen, sei aber nochmals (vgl. 2. Mitteilung, S. 126) betont, daß auch die letzten Ergebnisse immer wieder die volle Brauchbarkeit der Methode in ihrer bisherigen Form aufgezeigt haben.

Das Kaliummetall. Die Aufbewahrung der 120 bis 150 mg schweren Kaliumstücke erfolgt in Ligroin vom Siedepunkt 100 bis 120° C. Das dazu verwendete Ligroin wird zur Vorreinigung mehrere Wochen lang in einer Flasche über einigen Kaliumstücken aufbewahrt, die Flasche gelegentlich geschüttelt und das Ligroin schließlich durch ein trockenes Filter vom Schlamm abfiltriert, bevor es in Gebrauch genommen wird; Kalium, in derart vorbehandeltem Ligroin aufbewahrt, bleibt lange Zeit blank.

Kurz vor der Verwendung wird ein Kaliumstück in 5 ml Ligroin (diesmal vom Siedepunkt 55 bis 70° C) getaucht, dem 10 Tropfen Amylalkohol zugesetzt wurden. Nach etwa 1 Minute hat das Metall Hoch-

glanz angenommen. Das Ligroin-Amylalkoholgemisch wird in einem Wägegläschen geschlossen aufbewahrt und bleibt so für viele Analysen gebrauchsfertig.

Der Aufschluß. Der Aufschluß mit metallischem Kalium ist nach unseren Erfahrungen viel weniger schwierig als manch andere Operation im mikroanalytischen Laboratorium; trotzdem scheint der Analytiker anfänglich geneigt zu sein, diesem Teil der Methode die größte Aufmerksamkeit zu widmen und hier allein Gründe für das Versagen einer Analyse zu suchen.

Die Aufschlußart ist genau beschrieben worden (2. Mitteilung, S. 126 bis 129). Sie wird heute noch in derselben Weise unverändert durchgeführt, ohne daß sich eine Verbesserung als notwendig erwiesen hätte. Mit Mikro-Substanzeinwaagen und unter Anwendung von Kaliumstücken im Gewicht von 120 bis 150 mg, in Aufschlußröhrchen mit einer lichten Weite von 6 mm und bei einer Aufschlußtemperatur von etwa 800° C (Kalium*dämpfe*) ist die Aufschlußart so genau festgelegt, daß nicht mehr viel Spielraum für Variationen übrig bleibt. Das Kalium ist dabei auf ein Glasstäbchen aufgespießt, das nicht kürzer als 25 mm sein soll.

Die Aufschlußdauer — gemeint ist damit der Zeitbedarf für das Herunterschmelzen des Kaliums bis zur vollendeten Reaktion mit der Substanz — beträgt zwangsläufig zwischen 2 und 4 Minuten.

G. Bussmann[2] und auch *G. Kainz*[5] haben diese Aufschlußart unverändert übernommen. *W. Kirsten* und *C. Carstens*[3] dagegen konnten nur schlechte Ergebnisse erzielen; sie variierten deshalb ihre Arbeitsweise, indem sie es mit „sehr viel" bzw. „sehr wenig" Kalium versuchten, den Aufschluß sehr schnell oder sehr langsam durchführten, um schließlich Temperatur und Erhitzungsdauer derart zu steigern, daß sogar die Wandung des Aufschlußröhrchens angegriffen wurde. Wir haben diese Variationen nicht vorgeschlagen und deshalb auch nie daraufhin geprüft, ob sie als Aufschlußverfahren zur Schwefelbestimmung überhaupt geeignet sind. Nach unseren bisherigen Erfahrungen möchten wir dies verneinen.

Die Destillation. Als Vorbereitung zur Destillation wird bekanntlich das überschüssig vorhandene Kaliummetall mit einigen Tropfen Methylalkohol aufgelöst*. *G. Bussmann*[2] fand, daß in besonders ungünstigen Fällen die Säure nicht bis zum Boden des Reaktionsröhrchens durchdringt, wodurch unzersetztes, schwefelwasserstoffhaltiges Kaliummethylat in dem Röhrchen verbleiben kann. Wir haben niemals solche Schwierigkeiten beobachtet, wenn der Methylalkoholzusatz auf 0,5 ml beschränkt blieb. Es bleiben dann immer noch genug Kaliumreste unzersetzt im Reaktionsröhrchen zurück, die beim Säurezusatz eine starke Reaktion und damit eine Vermischung bewirken.

* Vgl. 1. Mitteilung, S. 29: „Es ist erwünscht, den Methylalkoholzusatz gering zu halten. In der Regel kommt man mit 0,5 ml Methanol aus."

Der Vorschlag *G. Bussmanns* geht dahin, das Aufschlußröhrchen in drei gleiche Teile zu zerschneiden, sie in ein erlenmeyerförmiges Zersetzungskölbchen zu legen und die Säure zuzugeben, ohne daß vorher der Überschuß an Kalium mit Methylalkohol beseitigt wurde. Wir wollen hierzu nicht unsere Bedenken verhehlen, daß das Zerschneiden des Reaktionsröhrchens die Gefahren in sich birgt, daß an den Bruchstellen des Glases Kalium-Kaliumsulfidgemisch verlorengehen oder oxydiert werden kann.

Die Form des *Quarzzersetzungskölbchens* des Destillationsapparates ist geändert worden. Trotz des am Boden 10 mm hoch angeschmolzenen Quarzgrieses und der seitlich anzusetzenden Mikroflamme des Brenners erforderte der Siedevorgang ein gewisses Maß an Aufmerksamkeit, darauf gerichtet, Siedeverzüge zu verhindern.

Abb. 1. Quarzzersetzungskölbchen, neue Form, mit eingelegtem Aufschlußröhrchen.

Abb. 1 zeigt die neue Form, in der ein ruhiges Sieden gewährleistet wird. Quarzkölbchen der neuen Form wurden von der Fa. Paul Haack, Wien, vorgeschlagen. Ältere Kölbchen ohne diese Siedeerleichterung können durch Einbuchten der Quarzwandung vor der Sauerstoffflamme zur neuen Form umgewandelt werden. Das Anschmelzen von Quarzsplittern am Kölbchenboden wurde trotzdem noch beibehalten.

Der einfache Hahn an der Wasserstoffzuleitung des Destillierapparates wurde von *P. Haack* durch einen Patenthahn ersetzt; es wird dadurch erreicht, daß während der Salzsäurezugabe in das Quarzzersetzungskölbchen der Wasserstoffstrom abgestellt bleibt.

Die Oxydation des Cadmiumsulfids in der Jodid-Jodat-Lösung. Wir konnten früher schon nachweisen (3. Mitteilung, S. 81), daß Cadmiumsulfid in natriumacetathaltiger, wässeriger Lösung durch neutrales Jodid-Jodatgemisch nicht oxydiert wird. Weiter konnte gezeigt werden, daß durch vollständiges Ansäuern eines solchen Gemisches mit Salzsäure die Oxydation des Sulfids zu Schwefel durch das in statu nascendi freiwerdende Jod so schnell erfolgt, daß Schwefelwasserstoff aus der Lösung nicht entweichen kann, eine Erkenntnis, die die Einsparung der Bremskapillare ermöglichte. Die daraus resultierende Arbeitsweise ist genau beschrieben worden (3. Mitteilung, S. 82) und bedarf eigentlich keiner Ergänzung mehr. Trotzdem sei auf eine Fehlermöglichkeit hingewiesen, die bei unvorschriftsmäßiger Anwendung der Methode an dieser Stelle zu falschen (zu hohen) Ergebnissen führen kann.

Nach dem Ablassen des Cadmiumsulfidniederschlages in die neutrale Kaliumjodid-Kaliumjodatlösung und Spülen der Absorptionsvorlage

mit Wasser wird zur quantitativen Erfassung der letzten Spuren Sulfid in der Vorlage, diese nochmals mit Wasser gefüllt, mit 0,5 bis 1,0 ml n/50 Kaliumjodatlösung, einigen Tropfen 10%iger Kaliumjodidlösung und anschließend sofort mit 20 Tropfen verd. Salzsäure (1 : 1) versetzt (= Reinigungsjodlösung). Diese Reinigungsjodlösung wird nach quantitativer Entfernung von Sulfidspuren aus Absorptionsvorlage und Einleitungsrohr ebenfalls in das Erlenmeyerkölbchen einlaufen gelassen.

Die Reinigungsjodlösung soll *schnell* in das Erlenmeyerkölbchen einlaufen. Zum unverzüglich anschließenden Spülen der Absorptionsvorlage *und* gleichzeitig zur quantitativen Umsetzung der Jodid-Jodatlösung sollen 20 ml Salzsäure (1 : 1) genommen werden (3. Mitteilung, letzter Abschnitt). Es wird damit erreicht, daß die basische Lösung von Natriumacetat-Cadmiumacetat-Kaliumjodid-Kaliumjodat ($p_H \sim 8$), in der sich der Cadmiumsulfidniederschlag unbegrenzt lange Zeit hält, so schnell wie möglich in die saure Jodlösung ($p_H \sim 1{,}2$) übergeführt wird, in der die Reaktion $CdS + J_2 = CdJ_2 + S$ glatt verläuft*.

Durch die Zugabe der Reinigungsjodlösung wird die Wasserstoffionenkonzentration von p_H 8 zunächst nur auf etwa 5,5 gesenkt. In diesem Zwischenstadium kann durch die Bildung von Hypojodit ein anderer Oxydationsverlauf möglich sein. Wir haben schon früher gefunden, daß bei Anwendung von *Mikro*mengen Schwefel der Ablauf der Reaktion $CdS + J_2 = CdJ_2 + S$ durch das kurzzeitige Verbleiben im p_H-Bereich 5,5 bei normalem Arbeitstempo keineswegs gestört wird. Wir haben aber auch damals gesehen, daß mit steigenden Mengen Schwefel der Einfluß unkontrollierbarer Nebenreaktionen anwächst. Diese Erkenntnis war mit einer der Gründe, warum wir den Wunsch, diese Mikromethode zu einer Halbmikro- oder Makromethode zu erweitern, noch nicht erfüllen konnten.

Die Höhe der Einwaage hat sich nach dem Verbrauch an n/50 Kaliumjodatlösung zu richten. Mehr als 8 ml sollen nicht verbraucht werden (3. Mitteilung, S. 81). Damit werden Schwefelmengen bis zu 2,56 mg S, entsprechend 6 mg Thioharnstoff oder 14 mg Sulfanilsäure, noch quantitativ erfaßt. Für die Praxis hatten wir 3 bis 10 mg Substanz (je nach Schwefelgehalt) vorgeschrieben (1. Mitteilung, S. 33).

Die Titration. Die jodometrische Titration des Cadmiumsulfids ist sehr exakt. Selbst Bruchteile eines Tropfens aus einer Mikrobürette gestatten, den Endpunkt einer Titration zweifelsfrei festzustellen. Wir gehen jetzt in ähnlicher Weise vor, wie es von *Pregl* für die Mikro-Säuretitration empfohlen wurde.

Der Jodüberschuß wird in langsamer Tropfengeschwindigkeit mit n/50 Thiosulfatlösung wegtitriert. Kurz vor dem Endpunkt werden

* Deshalb ist darauf zu achten, daß der Flüssigkeitsablauf aus der Absorptionsvorlage durch kapillare Verengungen im Hahnstopfen oder Einleitungsrohr nicht verzögert wird.

einige Körnchen Stärke oder einige Tropfen frisch bereitete Stärkelösung zugegeben; die Titration wird langsam fortgesetzt, bis die Titrierlösung durch *einen* Tropfen Thiosulfatüberschuß farblos geworden ist, worauf sofort wieder 1 bis 2 Tropfen n/50 Jodatlösung zugesetzt werden.

Die jetzt wieder blaue Lösung bleibt nun 7 Minuten im verschlossenen Erlenmeyerkölbchen stehen. Es ist dies die Zeit, die für das Nachlaufen der Lösungen in den Mikrobüretten notwendig ist. Diese Zeit wird aber auch gebraucht, um eventuell in dem Schwefelpräzipitat eingeschlossene Spuren von Jod in Freiheit zu setzen. Die Blaufärbung der Lösung kann sich daher in dieser Wartezeit etwas vertiefen. Nach Ablauf der Wartezeit wird die Titrierlösung wiederum durch Zusatz von 1 bis 2 Tropfen n/50 Thiosulfatlösung entfärbt. Unmittelbar anschließend erfolgt die Feintitration mit *gemessenen* Mengen von jeweils genau 0,02 ml n/50 Kaliumjodatlösung. Es wird also nicht mehr „auf den Tropfen" titriert, da die Tropfengröße eine von der Ausflußöffnung der Bürette und der Abfallgeschwindigkeit des Tropfens abhängige variable Größe darstellt. Beim Zusatz der letzten 0,02 ml Lösung wird der Bürettenhahn langsam zur Stellung „Auf" gedreht, während man gleichzeitig mit der Lupe den Meniskus der Jodatlösung so lange beobachtet, bis er um 0,02 ml gesunken ist. Nach dem Schließen des Hahnes wird der entstandene „Mikro"-Tropfen an der Wandung des Titrierbechers *oberhalb* der Flüssigkeitsoberfläche flüchtig abgestreift; durch Neigen des Titrierbechers wird der Tropfen mit der Flüssigkeit vereinigt. Die entstandene hellblaue Färbung bleibt eine Stunde lang völlig unverändert.

Prüfung auf Blindwert und Reinheit der Reagenzien. Die Methode weist weder einen positiven noch negativen Blindwert auf, sofern die Reagenzien rein sind. Es ist dabei zu unterscheiden zwischen Verunreinigungen, durch die zusätzlich während der Analyse Sulfid erzeugt werden kann, und solchen Verunreinigungen, die bei der jodometrischen Bestimmung des Sulfids zusätzlich Jod verbrauchen oder Jod erzeugen können.

Der erstere Fall kann nur eintreten bei Verwendung von unreinem Kaliummetall oder einer (in bezug auf Schwefelgehalt) schlechten Glasqualität der Aufschlußröhrchen*. Die Prüfung auf Reinheit erfolgt in diesem Fall durch eine Blindwertanalyse, wobei man durch Verwendung von möglichst viel Kalium (150 mg) und Anwendung einer höheren als der normalen Aufschlußtemperatur den ungünstigsten Grenzfall zu finden sucht. Die Praxis hat gezeigt, daß ein positiver Blindwert aus dieser Quelle nur selten auftritt. Trotzdem sollte bei jedem Wechsel speziell des Kaliumvorrates darauf geachtet werden.

Der zweite Fall kann eintreten, wenn die Reagenzien Natriumacetat-Cadmiumacetatlösung, Kaliumjodid oder Salzsäure unrein sind und ent-

* Gute Glasqualitäten sind Jenaer Geräteglas und Pyrexglas.

weder Jod verbrauchen oder Jod in Freiheit setzen. Die Prüfung auf Reinheit in diesem praktisch schon beobachteten Fall ist einfach: Man vermischt im Titrierkolben 10 ml Natriumacetat-Cadmiumacetatlösung, 2 ml 10% Kaliumjodidlösung und 20 ml verd. Salzsäure (1 : 1), verdünnt die Mischung mit 10 ml dest. Wasser (aus derselben Spritzflasche, aus der man bei der Analyse das Wasser zum Spülen entnimmt) und setzt Stärke in Form von Lösung oder fest zu.

Die Reagenzien sind rein, wenn nach 7 Minuten Wartezeit im geschlossenen Titrierkolben in der Lösung entweder eine Blaufärbung aufgetreten ist, die nach Zusatz von genau 0,02 ml n/50 Thiosulfatlösung wieder verschwindet, oder, sofern die Lösung farblos geblieben ist, nach Zusatz von 0,02 ml n/50 Kaliumjodatlösung eine Blaufärbung auftritt, die weitere 7 Minuten bestehen bleibt. Wir fanden gelegentlich, daß Kaliumjodid für eine zu starke Blaufärbung und Salzsäure für einen Jodverbrauch verantwortlich sein können. Es dürfte in einem solchen Fall stets richtiger sein, die Reagenzien auszuwechseln als einen Blindwert bei der Berechnung einzusetzen.

Nachtrag. Die Ergebnisse der von *W. Kirsten* und *C. Carstens*[3] mitgeteilten Nachprüfung der Methode machen es notwendig, auf deren Veröffentlichung einzugehen. Sie schreiben, daß bei Schwefelgehalten von etwa 40% bei Anwendung der ursprünglichen Methode (1. Mitteilung) mit Analysenfehlern bis zu 4% zu rechnen sei und durch Anwendung der neuen Vorschrift (gemeint ist die Arbeitsweise ohne Bremskapillare) der Fehler auf etwa 2% reduziert würde. Sie vermuten, daß sich beim Erhitzen der Substanz im Reaktionsröhrchen Kohlenstoffsulfide bilden, die durch Kalium und Salzsäure nicht zersetzt würden. Bei der neuen Arbeitsvorschrift werde der Fehler dadurch ungefähr kompensiert, daß eine Titrationsmethode benutzt wird, die zu hohe Werte gibt. Die Methode sei aber doch in Fällen, wo große Genauigkeit nicht notwendig ist, wegen ihrer Schnelligkeit und Einfachheit vor allem dann von großem Nutzen, wenn es gälte, aschehaltige Substanzen zu analysieren.

Wir sind dagegen der Ansicht, daß eine Methode mit einer derart hohen Fehlerkompensation als mikroanalytisches Verfahren zu verwerfen sei. Zur Entscheidung dieser Frage sind wir der Aufforderung von *Kirsten* und *Carstens* gefolgt, die ihre Notiz in der Absicht veröffentlicht haben, andere Laboratorien zu einer genaueren Überprüfung der Methode anzuregen.

Zu diesem Zweck haben wir eine Serie von je sechs Mikroeinwaagen von Sulfanilsäure und Thioharnstoff in unregelmäßiger Reihenfolge analysiert. Die Einwaagen wurden von einer zweiten Person ausgeführt und die Substanzgewichte erst nach Beendigung aller Analysen zur Vornahme der Berechnungen ausgehändigt. Der Aufschluß wurde unverändert nach der in der 2. Mitteilung (S. 126) gezeigten Arbeitsweise und die

Weiterverarbeitung gemäß 3. Mitteilung (S. 81, 82) ohne Verwendung einer Bremskapillare durchgeführt.

Ergebnis:

Nr.	Substanz*	Einwaage mg	n/50 Jodat ml	Gefunden % S	Δ%
1	T	5,245	6,924	42,32	+0,21
2	S	6,775	3,903	18,46	—0,05
3	S	5,971	3,451	18,52	+0,01
4	T	4,189	5,488	42,00	—0,11
5	S	5,630	3,214	18,30	—0,21
6	T	5,911	7,745	42,00	—0,11
7	T	5,237	6,891	42,18	+0,07
8	T	4,168	5,462	42,01	—0,10
9	S	4,861	2,790	18,40	—0,11
10	T	3,491	4,483	42,03	—0,08
11	S	3,840	2,211	18,45	—0,06
12	S	3,141	1,794	18,31	—0,20

* S = Sulfanilsäure (18,51% S), T = Thioharnstoff (42,11% S).

Kirsten und *Carstens* beweisen die Unbrauchbarkeit der Methode an Hand einer Reihe von „Modell"-Analysen, nachdem sie im normalen Analysengang mit Toluolsulfochlorid (16,81% S) Schwefelwerte zwischen 16,0 und 17,5% und mit Thioharnstoff sogar Streuungen zwischen 40 und 44% S feststellten.

Zur Klärung der Differenzen müßten die *Kirsten-Carstens*schen Modellanalysen in die Nachprüfung mit eingeschlossen werden. Dies ist aber nicht ohne weiteres möglich, da die genannten Autoren Arbeitsbedingungen gewählt haben, die weit über den Bereich einer vergleichbaren Mikroanalyse hinausgehen. Aus der von ihnen als Standard angewandten Natriumsulfidlösung entnahmen sie jeweils eine 6,4 mg Schwefel enthaltende Menge. 6,4 mg Schwefel entsprechen aber einer *Halbmikro*einwaage von etwa 38 mg Toluolsulfochlorid; sie verbrauchen 20 ml einer n/50 Kaliumjodatlösung, so daß eine mehrmalige Füllung einer Mikrobürette notwendig würde.

Wir sind der Meinung, daß die *Kirsten-Carstens*schen Fehlresultate bei Anwendung der mikromaßanalytischen Schwefelbestimmungsmethode noch durch ebenfalls im Mikromaßstab durchgeführte Modellanalysen ergänzt werden müßten, bevor in eine Diskussion über die ihnen möglicherweise unterlaufenen Fehler eingetreten werden kann.

Es ist mir eine angenehme Pflicht, Herrn *Heinrich Jerie* für die Durchführung der Analysenserie und für die Hilfe, die er mir bei der Weiterentwicklung der Methode geleistet hat, bestens zu danken.

Zusammenfassung.

Weitere Erfahrungen mit der mikromaßanalytischen Schwefelbestimmungsmethode werden mitgeteilt, aus denen sich die volle Brauchbarkeit dieser Methode in der früher mitgeteilten Form ergibt. Auf die verschiedenen Veröffentlichungen, die seither darüber erschienen, wird näher eingegangen. Angaben zur Vermeidung allfälliger Fehlermöglichkeiten werden gemacht.

Summary.

Further experiences with the microanalytical method for the determination of sulfur are reported. They show that this method is thoroughly usable in the form previously described. The publications that have appeared in the meantime are discussed. Statements are given regarding the avoidance of possible errors.

Résumé.

On communique de nouvelles expériences sur la microméthode de dosage du soufre, grâce auxquelles on se rend compte de la complète utilité de cette méthode sous la forme précédemment publiée. On se familiarise avec les diverses publications qui ont paru depuis sur le sujet. On donne des indications pour éviter les erreurs possibles dans tous les cas.

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Determination of Halogens.

Preliminary Report.

By

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With 1 figure.

(Received September 4, 1952.)

In the determination of halogens by dry combustion it is important to ensure complete absorption of the combustion products and to obtain the resulting halogens in a high concentration with a minimum amount of foreign ions. This is achieved with the apparatus showed in Figure 1. Oxygen is allowed to pass into the combustion tube through opening *A* and hydrogen through *B* for a few minutes in order to fill the connections and vessels with the pure gases. Stopcock *C* is then closed and the electric furnaces are heated; split-type sample-heater *D* to 700° C, combustion and hydrogenation furnace *E* to 850° C and split-type heating furnace *F* to 550° C. When the furnaces are hot the boat with sample *G* is introduced through *A* and stopcock *C* is opened. The rate of the oxygen flow depends upon the size of the apparatus and the sample. The rate of the hydrogen flow is adjusted so that almost all hydrogen reacts with the oxygen to form water and almost no gas passes through capillary *H*. One drop of sodium hydroxide solution is placed in vessel *K* in order to avoid losses of halogen by volatilization. Furnace *D* is drawn over the tube and the moving motor is switched on. The next sample may now be weighed out. The sample is burned in section *L*. In section *M* all halogens are reduced to the hydrides and these are immediately absorbed in the water which is formed at the same time and which slowly passes out through capillary *H*. Small amounts of ammonium halides are precipitated in *H* at the exit end of furnace *F*. When the combustion is finished furnace *F* is pushed back from the tube and copper cooler *N* is placed on this part of capillary *H*. The water formed in the reaction is now already condensed in section *O* and washes out the ammonium salts which were deposited there. Section *O* is

made of opaque quartz in order to avoid radiation of heat inside the quartz-walls. Vessel *K* is now lowered and the tip of capillary *H* is washed with a drop of water. The titration can be carried out immediately in the few drops of solution in vessel *K* with a suitable ultramicroburette. Stopcock *C* is then closed and the next sample is introduced through *A* and analyzed in the same manner etc.

Nitrogen does not interfere. Sulphur forms small amounts of sulphide which must be removed before the titration. This is conveniently done in the following manner: The solution is heated to boiling and one drop

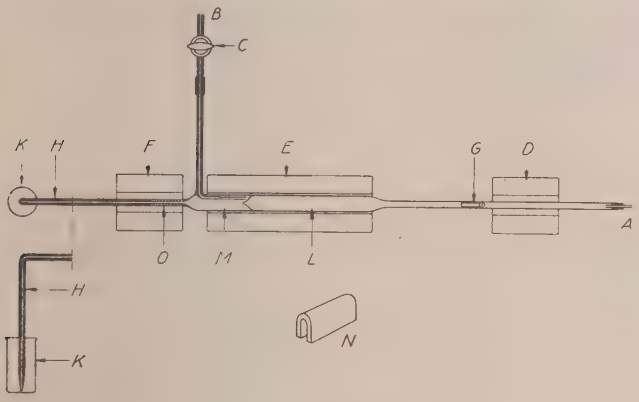

Fig. 1.

of 30% hydrogen peroxide is added. The solution is then kept boiling for further two minutes and cooled. As the concentration of the halogens is as high as in ordinary macrodeterminations a *Volhard*titration is quite convenient for samples between 2 and 5 mg. In samples down to 0.5 mg, halogens have successfully been determined potentiometrically using the electrode system of *Clark*[1] according to the method of *Cunningham, Kirk* and *Brooks*[2]. A paper will be published later.

Summary.

In the determination of halogens by dry combustion, complete absorption and a high concentration of the resulting halogens, with a minimum amount of foreign ions, is obtained by allowing the oxygen and the combustion products to react with hydrogen. Water is thus formed and the resulting halogen hydrides are absorbed in this.

Zusammenfassung.

Bei der Bestimmung von Halogenen durch trockene Verbrennung erhält man die Halogene in einer hohen Endkonzentration mit einem Minimum an

Fremdionen, wenn man den überschüssigen Sauerstoff und die Verbrennungsprodukte mit Wasserstoff reagieren läßt. Dabei entsteht Wasser, in dem sich die gebildeten Halogenwasserstoffe lösen.

Résumé.

Dans le dosage des halogènes par combustion sèche, on obtient une absorption complète et une concentration élevée des halogènes résultants avec une quantité minimum d'ions étrangers, en laissant l'oxygène et les produits de combustion réagir avec l'hydrogène. Il se forme ainsi de l'eau et les hydracides halogénés formés s'y dissolvent.

Literature Cited.

[1] *W. Clark*, J. Chem. Soc. London **1926**, 749.

[2] *B. Cunningham, P. L. Kirk* and *S. C. Brooks*, J. Biol. Chem. **139**, 11 (1941).

From the Direccion Nacional de Química, Rosario, Argentina.

Kjeldahl Method for the Semimicro Determination of Nitrogen in Beer.

By

Alberto J. Llacer*.

(Received May 25, 1952.)

Marcali and *Rieman*[1], described a Kjeldahl procedure in which the distillation of the ammonia is eliminated. The acid digest is neutralized with the use of methyl red whereupon the solution is treated with formaldehyde and titrated with alkali to the pink color of phenolphthalein. Since the liberated ammonia is immediately converted to the very weak base hexamethylenetetramine, the titrated solution will assume a p_H greater than 8 only upon addition of a slight excess of strong alkali after complete decomposition of the ammonium salt.

The same authors state[2] that they successfully developed micro and semimicro modifications for samples free from phosphorus, but that no advantage is gained by eliminating the distillation of ammonia which may be very rapidly performed on the small scale. It has been found, however, that the resulting simplification of apparatus is a desirable feature for routine work. Thus, a semimicro modification is described, which has been found practical for the determination of nitrogen in beer.

The findings of *Patel* and *Sreenivasan*[3] have been considered in establishing the conditions for the acid digestion in presence of potassium sulfate, mercuric oxide, and selenium. The mixed indicator of *Ma* and *Zuazaga*[4] was chosen in the place of methyl red, since the final end point after addition of phenolphthalein is more sharply defined: a certain intensity of the pink coloration produced by the presence of phenolphthalein compensates the green alkaline color of the mixed indicator to render the titrated solution nearly colorless or light gray. This is taken for the phenolphthalein end point. The purple color of the phenolphthalein appears upon the addition of a small excess of alkali.

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Apparatus.

Automatic buret of 25- or 50-ml capacity and connected to a storage bottle so that the solution in buret and storage is protected against the carbon dioxide of the atmosphere. Storage bottle should be made of alkali-resistant glass.

Automatic buret of 10-ml capacity divided into 0.05 ml with alternate graduation marks extending to three-fourth of the circumference of the buret stem, connected to a reservoir so that the standard solution is protected against the carbon dioxide of the atmosphere.

Precision transfer pipet, 2.00-ml delivery.

Round-bottomed Kjeldahl flask, Pyrex, 100-ml capacity.

Erlenmeyer flask, 200-ml capacity.

Reagents.

Concentrated sulfuric acid, Baker's C. P., sp. gr. 1.84, 94–96.5 per cent.

Catalyst, mixture of 1 g elemental selenium, 10 g mercuric oxide, and 120 g potassium sulfate, all Baker's C. P. reagents, carefully ground and intimately mixed.

Hydrogen peroxide solution, 30 per cent Baker's C. P. perhydrol reagent is diluted with an equal volume of water just before use.

Sodium bromide solution, 150 g of Baker's C. P. reagent, containing 0.0005 per cent nitrogen, is dissolved in water to give 250 ml solution.

Formaldehyde solution, approximately 20 per cent. Just before use, 5 ml of Merck's reagent-grade formaldehyde, 38–40 per cent, are diluted with an equal volume of water; 5 drops of 0.003 molar phenolphthalein solution is added, and the mixture is titrated with 0.05 N sodium hydroxide to a just detectable pink color.

Sodium hydroxide solution, approx. 19 N, is prepared by dissolving 600 g of sodium hydroxide, Baker's C. P. pellets containing 0.001 per cent nitrogen, 0.005 per cent silica, and a like amount of oxides precipitated by ammonia, in 600 ml of water. The solution is allowed to stand for several days in a paraffin-lined and rubber-stoppered bottle. The clear supernatant solution is separated from the carbonate precipitate by decantation.

Sodium hydroxide solution, approximately 10 N, is obtained by diluting 800 g of the 19 N solution with carbon dioxide-free water to give 1 liter solution. The solution is stored in the storage bottle of alkali-resistant glass, which is connected to the 25- or 50-ml buret.

Standard sodium hydroxide solution, 0.05 N, is prepared by diluting with carbon dioxide-free water 4 g of the 19 normal solution to 1 liter. The solution is stored in the bottle of alkali resistant glass, which is

connected to the 10-ml buret. It is standardized against potassium biphthalate.

Sulfuric acid solution, approximately 0.3 N. Approximately 8.4 ml of the concentrated acid is added to 1 liter of water.

Methyl red-bromocresol green indicator solution contains 0.04 g methyl red and 0.02 g bromocresol green in 100 ml alcohol.

Phenolphthalein indicator solution, 0.003 molar, obtained by dissolving 0.25 g of the indicator in 250 ml alcohol.

Procedure.

Transfer a sample of exactly 2.00-ml of the beer to the 100-ml Kjeldahl flask. Place the flask on the digestion rack and gently heat with a micro flame until the volume of the sample is reduced to approximately 0.5-ml. Add 3 ml of concentrated sulfuric acid and about 300 mg of the catalyst. The catalyst may be conveniently measured by means of a glass tube into which a closely fitting glass rod is inserted to act as a piston. The tube is loaded by repeatedly pressing its end against the catalyst mixture until the tube is filled up to a predetermined mark. The glass rod serves to compact the mixture and, finally, to eject it.

Mix the contents of the Kjeldahl flask by swirling and then heat to a temperature below the boiling point of the mixture. When the frothing stops, raise the temperature to obtain a gentle boiling. When the mixture becomes nearly colorless, interrupt the heating and add approximately 0.5 ml of the hydrogen peroxide solution. Complete the digestion by heating for 15 to 20 more minutes.

When the digestion is completed, allow flask and contents to cool to at least 50° C and then add 5 ml of water in such a manner that the neck of the flask is carefully rinsed into the bulb. The contents of the Kjeldahl flask are then quantitatively transferred to an Erlenmeyer flask of 200-ml capacity. This may be efficiently done by means of a suction-operated syphon made of thick-walled capillary tubing. Rinsing with 5 or 6 portions of 5 ml of water will complete the transfer, if every portion of wash water is added in a manner assuring a thorough rinsing of the neck of the Kjeldahl flask.

Treat the contents of the Erlenmeyer flask with 1 ml of sodium bromide solution and 5 drops of the methyl red-bromocresol indicator mixture. To neutralize the solution titrate with 10 normal sodium hydroxide and 0.3 N sulfuric acid. When the solution contains a very slight excess of acid, gently boil for 1 to 3 minutes to expel the carbon dioxide. Then cool to room temperature and add sodium hydroxide until the color of the solution changes to yellow. Backtitrate with the acid to a pink color, and then titrate with 0.05 N sodium hydroxide standard solution to an

orange coloration which represents the end point color of the mixed indicator.

Treat the neutralized solution with 10 ml of the formaldehyde solution. The color of the solution to be titrated may change to pink. Take the zero reading of the 10-ml buret and then titrate with 0.05 N sodium hydroxide standard solution until the color of the solution in the Erlenmeyer flask changes from yellow to green. Add 5 drops of phenolphthalein indicator solution and continue adding sodium hydroxide standard solution until the green color fades to almost exactly colorless. This represents the end point of the titration of the ammonia. Thus, take the final reading of the buret. The volume of standard solution used is equivalent to the nitrogen present.

Results and Conclusions.

The precision of the method was tested by performing 10 determinations on 2.00-ml portions of one sample of beer. The results are given in Table I which indicates a standard deviation of $\pm$ 5 parts per thousand from the mean value.

Table I. Determination of Nitrogen in 2-ml Portions of a Beer Sample.

0.05 N NaOH ml	Deviations from Mean
2.97	0.01
2.97	0.01
2.98	0.00
3.00	0.02
2.98	0.00
2.97	0.01
3.01	0.03
2.98	0.00
2.97	0.01
Mean 2.98	standard deviation: $\pm$ 0.014 ml or $\pm$ 0.5%

The recovery of nitrogen was tested by adding known quantities of ammonium sulfate to beer. The ammonium sulfate was Baker's C. P., reagent grade, and it was standardized by titrating it with 0.05 N sodium hydroxide in presence of formaldehyde as outlined in the procedure. All results given in Table II are the means of quadruplicate determinations corrected for blanks carried out in duplicate. It may be seen that 98.5 per cent of the nitrogen is recovered on an average.

It is, of course, necessary to adhere closely to the prescribed concentrations and volumes of the various reagents including indicator solutions, which have been chosen after careful consideration of all requirements. Also the effects of variations in the procedure of digestion have been checked. Excessive heating during the initial stage of the digestion is not advisable. Prolonging the digestion beyond 15 to 20 minutes after adding the hydrogen peroxide does not affect the recovery of nitrogen. Significant losses were not observed even when this heating period was extended to 2 hours.

Table II. Recovery of Nitrogen Added to Beer in the Form of Ammonium Sulfate.

N added mg	N recovered		Deviation from theory %
	mg	%	
5.460	5.381	98.5	— 1.5
	5.369	98.3	— 1.7
4.970	4.899	98.6	— 1.4
	4.890	98.4	— 1.6
5.460	5.383	98.6	— 1.4
5.600	5.508	98.4	— 1.6
Average		98.5	— 1.5

When applying the described procedure to beer, the effect of the presence of phosphate is insignificant. A noticeable loss of nitrogen resulted, however, when zirconyl chloride was used as suggested by *Marcali* and *Rieman*[2] for the elimination of the phosphate interference.

Six determinations of nitrogen may be performed by one operator in somewhat less than 3 hours, but the number of simultaneous determinations performed in the stated time interval may be considerably increased after some practice. A Kjeldahl flask with removable neck as used by *Marcali* and *Rieman* permits a further saving of operator's time by eliminating the transfer of the digest to the Erlenmeyer flask.

Summary.

With some slight modifications, the method of *Marcali* and *Rieman* for the Kjeldahl determination of nitrogen without distillation of the ammonia may be advantageously used for the routine determination of nitrogen in 2.00-ml samples of beer.

Zusammenfassung.

Marcali und *Rieman* umgehen die Destillation des Ammoniaks bei Kjeldahlbestimmungen, indem sie den Aufschluß neutralisieren und dann in

Gegenwart von Formaldehyd mit Natronlauge bis zum Phenolphthaleinendpunkt titrieren. Da das freiwerdende Ammoniak augenblicklich mit dem Formaldehyd unter Bildung der sehr schwachen Base Hexamethylentetramin reagiert, kann man die als Ammoniumsalz gebundene Säure direkt titrieren.

Es wird gezeigt, daß sich das Verfahren von *Marcali* und *Rieman* vorteilhaft für Serienbestimmungen von Stickstoff in 2-ml-Proben von Bier verwenden läßt.

Résumé.

Marcali et *Rieman* évitent la distillation de l'ammoniaque dans le dosage Kjeldahl en neutralisant le produit d'attaque et le titrant avec la soude en présence de formaldéhyde jusqu'à virage de la phénolphtaléine. Du fait que l'ammoniaque libérée se combine immédiatement avec le formaldéhyde en donnant l'hexaméthylène tétramine, base très faible, on peut titrer directement l'acide combiné sous forme de sel ammoniacal. On montre que le procédé de *Marcali* et *Rieman* peut être avantageusement utilisé pour les dosages d'azote en série dans des échantillons de bière de 2 ml.

Literature.

[1] *K. Marcali* and *W. Rieman*, III, Ind. Engng. Chem., Analyt. Ed. **18**, 709 (1946).

[2] *K. Marcali* and *W. Rieman*, III, Analyt. Chemistry **20**, 381 (1948).

[3] *S. M. Patel* and *A. Sreenivasan*, Analyt. Chemistry **20**, 63 (1948).

[4] *T. S. Ma* and *G. Zuazaga*, Ind. Engng. Chem., Analyt. Ed. **14**, 280 (1942).

From the Direccion Nacional de Química, Rosario, Argentina.

Semimicro Pycnometer.

By

Alberto J. Llacer*.

With 1 figure.

(Received May 25, 1952.)

A Sprengel-Ostwald type pycnometer of approximately 2.5-ml capacity has been found very advantageous for precise routine determinations of density. The relatively large capacity makes it possible to perform the weighings on an analytical balance.

The pycnometer is made from a 50 cm length of Pyrex capillary tubing of 8 mm outside diameter and 0.7 mm inside diameter. Bulb G (Fig. 1) is blown 15 cm from one end of the tubing, and the safety bulb I at a distance of 4 cm from G. The latter is provided for the safety of the operator when working with liquids having a high vapor tension at room temperature.

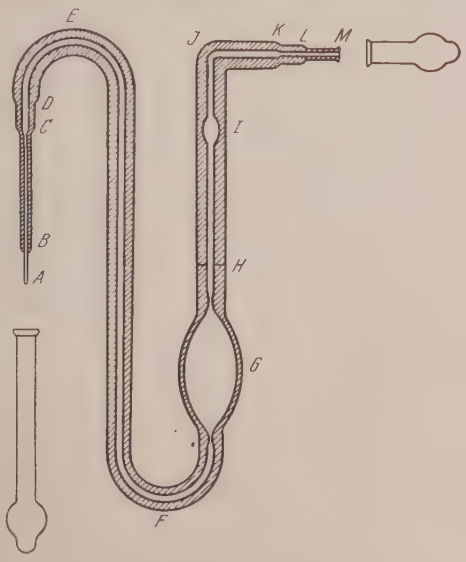

Fig. 1. Semimicro Pycnometer.

Section K to M is made by heating first at K. While collecting glass, a tiny bulb is blown, which is then drawn out to a length of 5 mm. The flame is then directed toward L, and a capillary of 0.5 mm inside diameter is

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drawn out, cut, and firepolished at M which is about 7 mm from L. Section D to A is obtained in a like manner. D is 17 cm from bulb G; the distance C–B is about 35 mm, and the capillary A–B is given a bore of 0.25 mm diameter.

The bends at J and F are made next. When finally making bend E, care must be taken to bring tip A to the same level as the mark H so that the hydrostatic pressure will be balanced when the pycnometer is filled. This as well as the constrictions at both sides of bulb G serves to give improved control in the operation of the apparatus.

The final task is the preparation of the caps and the grinding of the joints for which the sections C–D and K–L are to provide the necessary tapers.

For filling the pycnometer, M is connected with a rubber tubing of pure gum to a suitable aspirator. A magnifying glass is used to check the adjustment of the meniscus at mark H. If the meniscus should be slightly above the mark, touching filter paper to tip A will allow removal of the excess liquid. When the position of the meniscus is properly adjusted, the outside of capillary A–B is carefully wiped with filter paper. The contents of the pycnometer are inspected with the use of a magnifying glass to make certain that air bubbles are absent. Finally, the outside surface of the apparatus is wiped with chamois or linen cloth, and the openings are capped. For weighing, bend F is placed into the hook of the balance so that the pycnometer is suspended in an inverted position. A glass tare is used to counterpoise the empty pycnometer.

Table I. Weight of Carbon Dioxide-Free Water of $26.0 \pm 0.2°$ C Contained in the Pycnometer.

Weight of Water g	Deviation from Mean 10^{-5} g
2.49176	+ 4.6
2.49170	— 1.4
2.49172	+ 0.6
2.49171	— 0.4
2.49162	— 9.4
2.49171	— 0.4
2.49178	+ 6.6
Mean: 2.491714	standard deviation: $\pm 5.0 \times 10^{-5}$ g or $\pm$ 0.00002

Table I shows that a relative standard deviation of 1 part in 50000 may be attained in determining the density of water, if the temperature fluctuations of the liquid are kept within 0.5° C. The weighings were

performed on an Ainsworth analytical balance which allowed reproduction of weighings within $\pm$ 0.006 mg. A more exact control of the temperature as used by *Anderson*[1] for his automatically adjusting micropycnometers would probably permit increase of the precision.

Zusammenfassung.

Das in der Abbildung gezeigte Pyknometer von etwa 2,5 ml Inhalt eignet sich für genaue Serienbestimmungen. Unter Benützung einer guten analytischen Waage und einer Glastara für das leere Pyknometer kann der relative mittlere Fehler auf 1 Teil in 50000 gebracht werden, wenn die Temperatur der Flüssigkeit innerhalb 0,5° C konstant gehalten wird.

Résumé.

Le pycnomètre de 2,5 ml de capacité décrit sur la figure convient pour les déterminations précises en série. Par emploi d'une bonne balance de précision et d'une tare de verre pour le pycnomètre vide, on peut amener l'erreur relative moyenne à 1/50.000 quand la température intérieure du liquide est maintenue constante à moins de 0,5° C près.

Literature.

[1] *H. H. Anderson*, Analyt. Chemistry **20**, 1241 (1948) and **24**, 579 (1952).

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Determination of Lipids. A Density Gradient Method.*

By

Ahron Gibor and Paul L. Kirk.

(Received July 17, 1952.)

The use of density gradients for rapid comparison of the density of solids and liquids and for quantitative study of density changes has been extensively developed in recent years. The method has been applied to a number of qualitative identifications and comparisons of various solids by *Kirk* and coworkers[1]. Quantitatively, gradient tubes were used by *Linderstrøm-Lang*[2] for the estimation of enzyme activity in small aqueous drops suspended in a water-immiscible medium having a density gradient. The change in the density of the drop due to hydrolysis of the substrate by the enzyme caused a corresponding movement of the drop in the gradient tube. The rate of movement of the drop was correlated with the enzyme activity.

Density gradient tubes containing water-immiscible organic solutions were also used by *Lowry* and *Hunter*[3] for determination of the density of plasma and serum, which can be correlated with the concentration of dissolved proteins. The density of the suspended plasma drops was determined by reference to a set of drops of aqueous salt solutions of known density.

In the present study, the amount of dissolved fat in a heavy organic solvent was determined by measuring the density of the solution. Since the density of fats is less than 1.0, their solutions in a solvent such as chloroform will have a lower density than the pure solvent. To determine the density difference, sensitive density gradients consisting of liquid in which both the organic solvent and the fat are insoluble are required.

* This investigation was supported in part by Veterans Administration, under contract No. V 100 1M-1979, the National Cancer Institute of the National Institutes of Health, Public Health Service, and by the Committee on Research of the University of California.

Such gradients were prepared from strong aqueous solutions of heavy metal salts. Chloroform solutions of known concentration of pure fats were used as standards by suspending them in the density gradient tubes. The density of any sample could then be determined by introducing a drop of it in the same tube. The concentration of fat in the sample was evaluated by comparing the equilibrium level of the sample drop with that of the standard fat solutions. No correction for temperature variation was necessary because the standard drops responded to the same temperature change. The same spontaneous adjustment of standard drops occurred with the continuous slow change in the density due to the diffusion and equilibration of the different concentration levels of the tube.

Experimental.

Reagents.

1. *Dyed chloroform* was prepared by dissolving a small amount of Scharlach R in chloroform. The introduction of dye into the chloroform facilitated the observations on the small drops of solution in the gradient tube.

2. *Standard solutions* were prepared with weighed quantities of Neo Fat 3 R (Armour linoleic and oleic acids) dissolved in known volumes of the dyed chloroform. By appropriate dilution any strength of standard could be obtained.

3. *Cadmium chloride solution*, saturated, was prepared by dissolving as much cadmium chloride as possible in water and filtering the solution. This was diluted to the various concentrations needed for the gradient preparation, each of the diluted solutions being saturated with chloroform.

Apparatus.

Well-cleaned 50-ml burets were used as gradient tubes. The calibration marks served to identify levels in the gradient and the size of the tubes was such that drops could pass without touching the walls or each other, the former sometimes causing sticking to the wall and the latter allowing coalescence. Thorough cleaning was necessary to avoid attachment of the drops to the walls.

Drops were added to the gradient from small capillary pipets attached to syringe controls[4]. A water thermostat was advantageous especially when the room temperature was quite variable, but not essential for the most sensitive gradients.

Gradient preparation.

In order to prepare solutions that bracket closely the density of the chloroform solution of the fat, two solutions were prepared, one (solu-

tion A) with a density slightly greater than that of chloroform and the other (solution B) sufficiently lower in density to allow for the effect of any fat concentration that would be encountered. From these stock solutions, all gradients could be prepared, but for convenience, a preliminary gradient was established to locate the necessary range.

Five milliliters of the saturated cadmium chloride solution was transferred to a centrifuge cone and a drop of chloroform added. Filtered distilled water was added in 0.25-ml portions until the density of the resulting aqueous solution was just equal to that of chloroform as determined by the movement of the drop. An extra 0.5 ml of water was then added to confirm that the resulting solution was lighter than the chloroform. Having established the ratio of water to saturated solution, larger quantities of solution A and solution B were prepared. From these, a number, usually six, of solutions were prepared as shown in Table I.

Table I. Dilutions of Stock Solutions.

Stock No.	Solution A ml	Solution B ml
1	6	1
2	5	2
3	4	3
4	3	4
5	2	5
6	1	6

The preliminary gradient was prepared by adding a layer of solution A to the bottom of a gradient tube. On top of this was carefully layered an equal volume of stock 1, followed by an equal layer of stock 2, and so forth until the last layer contained only solution B. To this tube were then added a drop of dyed chloroform, and a drop each of a series of fat solutions in dyed chloroform. Each drop came to rest at the interface separating the solutions of higher and lower density, and indicated the range of gradient necessary for the most sensitive determination. For any range of concentration more sensitive gradients were readily established. The solutions just above and just below the interface in question were mixed in appropriate amounts to form the intermediate gradient liquid. A layer of the next heavier solution below, and the next lighter one above the sensitive region were added to prolong the useful life of the gradient.

Measurement.

Gradients in the range desired were prepared as described above. The solutions were layered by adding them slowly from a buret or separatory funnel, allowing the solution to flow slowly down the side of the tube at a

rate of 2 to 3 ml/minute. A few drops of chloroform were added to the charged tubes which were then tightly stoppered and allowed to stand for 2 or 3 days before adding any samples, so that the gradient would become uniform.

Drops of standard fat solutions in chloroform were then added to establish a calibration. Each was introduced after inserting under the surface of the gradient liquid the tip of the pipet carrying the solution. The drop was forced out of the tip by means of the syringe control, and detached by a quick withdrawal of the pipet from the liquid. Drops of about 5 λ were conveniently delivered by this method. Following the standard drops, a drop of each of the sample solutions was inserted in the same manner. It was often necessary to insert the first drop into the preliminary gradient to ascertain that the density range was correct in the sensitive gradient. After reaching equilibrium in the sensitive gradient, the fat content of the unknown samples was evaluated by reference to the calibration droplets.

Recovery of Gradients.

After a tube contained as many drops as convenient, the gradient could be cleared of all added drops and recovered for further use by immersing it in a bath of warm water. The chloroform drops immediately rose to the surface and could be removed with a pipet. The gradient was immediately allowed to cool to room or thermostat temperature and could be used again with very little change from its original density levels.

The use of a thermostat was not essential when the room temperature was rather uniform, but the more sensitive the gradient, the more difficult became the control of temperature. A simple method of operating without a thermostat was to encase the gradient tubes in larger glass tubes filled with water. These had the effect of increasing the heat capacity and preventing rapid temperature changes.

Results.

It was soon noted that temperature had a very considerable effect on the levels assumed by the droplets, as expected because of the greater coefficient of expansion of chloroform than of water. Whereas this effect would be nullified by the simultaneous movement of the standard drops, it was necessary that all of the drops remain in the servicable portion of the gradient. The phenomenon was studied in a gradient of relatively low sensitivity, and over a period of 17 days for one set of drops, the data being shown in Table II. The effect of removing the drops by the method given and replacing with a new set of standards is also shown, the total test period being 33 days. Sensitivity was calculated in terms of the

difference in fat concentration represented by a movement of 0.05 large scale division on the buret tube, or 0.5 small division. While a buret reading may be estimated somewhat closer than this, a reading of this magnitude may be made very exactly, and the actual sensitivity figures are somewhat greater than this limit of estimation.

Table II. Positions of Drops as a Function of Concentration, Temperature, and Time.

Days	Temp.	Lipid Concentration in Chloroform				Sensitivity
		Zero	5.64 mg/ml	11.28 mg/ml	16.92 mg/ml	mg/ml
7	24	36.60	35.60	34.45	33.20	0.25
8	23	37.05	36.10	34.95	33.45	0.23
10	20	38.00	37.05	35.95	33.85	0.20
10	25	36.80	35.90	34.30	31.00	0.14
13	27	35.80	34.60	32.90		0.19
17	23	36.20	34.75	32.85		0.17
*21	21	37.60	36.65	35.30	34.20	0.25
24	20	37.75	36.80	35.55	34.45	0.25
*26	19	38.00	36.85	35.70	34.85	0.26
28	19	37.90	36.75	35.60	34.75	0.26
33	19	37.70	36.50	35.30	34.50	0.26

* Drops replaced by a new set.

While the effect of temperature is considerable, it causes no serious difficulty in any normal working range. The data also show clearly the great stability of the gradients. These did lose sensitivity to a measurable extent in a month but were still servicable over that period and allowed changes of sample without destruction of the gradient.

Table III. Positions of Drops as a Function of Concentration, Temperature, and Time

Days	Temp.	Lipid Concentration in Chloroform					Sensitivity
		Zero	5.64 mg/ml	11.28 mg/ml	16.92 mg/ml	22.56 mg/ml	mg/ml
4	22	24.00	22.70	18.35	17.05	15.75	0.14
5	20	24.30	22.50	19.00	17.70	16.90	0.15
*6	19	25.30	24.45	23.10	18.80	17.45	0.13
8	19	25.20	23.65	19.45	17.70	15.80	0.12
*18		24.40	23.10	18.55	17.30	15.80	0.13
20	20	25.60	23.50	19.30	18.30	16.50	0.12
21	21	25.40	23.00	19.10	17.50	15.75	0.12
22	21	25.40	23.00	19.05	17.45	15.70	0.12
25	24	23.65	20.15	17.30	15.50		0.10
*27	20	25.50	23.70		17.60	16.40	0.12

* Drops replaced by a new set.

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In Table III are shown the data obtained from a similar study with a gradient having approximately twice the sensitivity of the first. Again it is seen that the gradient was approximately constant in sensitivity for the 27 days during which it was tested. This gradient also was cleared three times of test and standard droplets and recharged. Again, the sensitivity dropped a little, but the difference is less striking than the constancy maintained by the gradient.

A major problem to be considered in the application of this procedure is the choice of a suitable fat for use in the standard drops since the densities of lipid mixtures from two sources may be different. This may best be solved by preparing a larger standard lipid mixture by extraction of an organ or other source similar to the unknown that is to be determined and using the large supply of lipid obtained to make the standards. When only neutral fat is to be studied, this precaution is not believed necessary since all neutral fats have quite similar densities. Even if the standard has a somewhat different density than the unknown, all values obtained relative to the standard will be comparable.

Another possible problem arises from the possibility of reaction between the lipid and the salt of the gradient. Thus, lecithin forms a cadmium salt. Some other salt which has a high enough density and solubility may be employed to form the gradient. While the choice is limited, it is probably sufficiently broad to allow application of the method under nearly all circumstances.

Summary.

A method is described by which chloroform droplets containing dissolved lipids may be analyzed for their lipid content by comparison with similar solutions in a density gradient tube with aqueous cadmium chloride solution. The procedure offers a very simple way in which lipids may be determined regardless of their source. The absolute limit of detection was of the order of 0.15 micrograms.

Zusammenfassung.

Die Konzentration in Chloroformtröpfchen gelöster Lipide kann durch Vergleich der Dichte dieser Lösungen mit jener von Lipidlösungen bekannter Konzentration ermittelt werden. Zum Dichtevergleich nach der Schwebemethode dient eine 50-ml-Bürette, die mit einer Cadmiumchloridlösung gleichmäßig abfallender Konzentration gefüllt ist. Lipide irgendwelcher Herkunft können auf diese einfache Art bestimmt werden. Die absolute Erfassungsgrenze beträgt 0,15 γ.

Résumé.

La concentration des lipoïdes dissous dans des gouttelettes de chloroforme peut être déterminée par comparaison de la densité de ces solutions

avec celles de solutions de lipoïdes de concentration connue. Pour la comparaison de densité par la méthode d'immersion, on se sert d'une burette de 50 ml remplie d'une solution de chlorure de cadmium s'écoulant régulièrement. Les lipoïdes de n'importe quelle origine peuvent être simplement déterminés par ce procédé. La sensibilité absolue atteint 0,15 γ.

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Die mikroacidimetrische Titration schwacher Säuren mit Phenolphthalein.

Von

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Sowohl bei der Mikroäquivalent-(Carboxyl-)Bestimmung wie auch insbesondere bei der Bestimmung von Acetyl-, Benzoyl- oder C-Methylgruppen wird die Säure, sofern ein acidimetrisches Bestimmungsverfahren gewählt wird, mit Phenolphthalein als Indikator titriert. Der Kohlensäureeinfluß wird dabei in bekannter Weise durch Aufkochen der sauren Lösung kurz vor dem Äquivalenzpunkt und Beendigung der Titration in der Siedehitze ausgeschaltet.

Man sollte annehmen, daß bei der Mikrotitration wegen der sicheren Feststellung des Phenolphthaleinumschlages eine genaue Erfassung des Endpunktes möglich sei. Eigene Erfahrungen wie auch ein kurzer Blick in die diesbezügliche Literatur zeigen aber, daß dem nicht so ist. *Pregl-Roth*[1] empfehlen, bis zur ganz schwachen Rosafärbung zu titrieren, die „wenigstens mehrere Sekunden bestehen bleiben soll". „Dem Anfänger, namentlich aber dem weniger Farbenempfindlichen bereitet diese Bestimmung Schwierigkeiten" (l. c. Seite 196). Die Titration ist beendet, „bleibt nach 3 Sekunden die Rosafarbe noch bestehen" (l. c. S. 197). Nach *E. Wiesenberger*[2] aber ist der Endpunkt der Titration erst erreicht, wenn eine schwache Rosafärbung 10 bis 15 Sekunden bestehen bleibt.

Erklärungen für dieses Verhalten des Indikators in der Siedehitze werden nicht mitgeteilt. Vermutlich sind sogar Einflüsse verschiedener Art wirksam. Beispielsweise sind ein erneuter Zutritt von Kohlensäure

und die Verschiebung des Umschlagintervalls von Phenolphthalein bei sinkender Temperatur Einflüsse, die entgegengerichtete Wirkungen auslösen können.

Die Titration sollte besser bei Zimmertemperatur beendet werden, vorausgesetzt, daß auch dann noch Kohlensäurefreiheit im Rahmen der normalen Fehlergrenze einer Mikrotitration aufrechterhalten bleibt. Bei der Titration ist mit dem Kohlensäureeinfluß aus zwei Quellen zu rechnen: Gasförmiges Kohlendioxyd aus der Luft und gebundenes Kohlendioxyd aus dem Karbonatgehalt der Titrierlauge herrührend. Beide Einflüsse werden bekanntlich durch Kochen der sauren Titrierlösung gleichzeitig ausgeschaltet.

Die Notwendigkeit, an diesem Punkt der Titration zu kochen, bleibt immer bestehen, aber die Titration muß deshalb nicht unbedingt in der Siedehitze zu Ende geführt werden. Es hat sich nämlich gezeigt, daß, wenn man die heiße Titrierlösung unter dem Schutz eines Natronasbeströhrchens abkühlt, wobei sie sich wieder mit (jetzt kohlensäurefreier) Luft sättigt, die kalte Titrierlösung nur sehr langsam Kohlensäure wieder aufnimmt. Auch die über der Titrierlösung im Erlenmeyerkölbchen stehende Luft ist kohlensäurefrei und wirkt als Puffer, so daß jetzt über die Dauer einer langsam durchgeführten Titration in der Kälte die Aufnahme von Kohlensäure ausgeschlossen ist. Versuche haben gezeigt, daß die schwache Rosafärbung einer kalt austitrierten Lösung beim Stehen im offenen 100-ml-Erlenmeyerkölbchen nach 30 Minuten noch nicht verblaßt ist*.

Durch Anwendung karbonatfreier, nach *Pregl* aus Öllauge hergestellter n/100 Natronlauge ist der Einfluß durch Karbonatkohlensäure von vornherein ausgeschaltet, theoretisch sind dann Faktor „heiß" und „kalt" gleich; bei Anwendung karbonathaltiger Natronlauge aber sind die Faktoren nicht mehr gleich. In der Praxis wird die Lauge immer einen geringeren Karbonatgehalt aufweisen. Bei der Feststellung des Endpunktes einer Titration mit karbonathaltiger Lauge muß deshalb die Zugabe der Lauge auf wenige Tropfen in der Kälte beschränkt bleiben, um den dadurch hervorgerufenen Fehler unterhalb der zulässigen Grenze zu halten. Die folgende Überschlagsrechnung zeigt, daß diese Forderung leicht erfüllt werden kann: Angenommen eine Natronlauge hat, in der Siedehitze mit n/100 Säure titriert, den Faktor 1,000. Ein hoher Anteil des Natriumions, nämlich 1/10 Val, sei dabei als Karbonat gebunden. Diese Lauge, in der Kälte titriert, zeigt dann einen um 5% gegenüber der Heißtitration veränderten, schwächeren Wert. Die zur Feststellung des Äquivalenzpunktes in der Kälte verbrauchte Natronlauge ist deshalb

* Alle Titrationen sollten natürlich nicht in allzu großer Nähe von Kohlensäurequellen (Bunsenbrenner) ausgeführt werden.

ebenfalls um 5% falsch. Da man aber für diesen Zweck höchstens fünf Tropfen Lauge (= 0,15 ml) verbraucht, beläuft sich der Fehlbetrag auf weniger als 0,01 ml n/100 Lösung.

Arbeitsweise: Die zu titrierende Säure wird in einem 100-ml-Quarzerlenmeyerkölbchen unter Anwendung des von *G. Kainz*[3] vorgeschlagenen Kühlzapfens 30 Sekunden gekocht, der Kühlzapfen mit 2 bis 3 ml heißem Wasser abgespült und die Lösung in der Hitze, nach Zusatz von *nur einem* Tropfen 1%iger Phenolphthaleinlösung bis kurz vor das Auftreten oder bis zum Auftreten der ersten, nur wenige Sekunden bestehen bleibenden Rosafärbung titriert. Dann wird der Titrierkolben sofort mit einem Natronasbestrohr*) verschlossen und unter dem Wasserstrahl gekühlt; man achte darauf, daß auch der Kolbenhals Zimmertemperatur angenommen hat. Nach dem Kühlen ist eine in der Hitze eventuell gerade noch erkennbar gewesene Rosafärbung in ein kräftiges Rot übergegangen bzw. eine in der Hitze noch farblos gewesene Lösung kann eine Rosafärbung angenommen haben (Verschiebung des Umschlagsintervalls des Indikators).

Die kalte Titrierlösung wird langsam unter mäßigem Schütteln des Titrierkolbens austitriert. Ein mehrmaliges Prüfen des Endpunktes durch tropfenweises Zugeben von Säure oder Lauge ist ebenfalls möglich, nur sollte der Gesamtverbrauch an Natronlauge bei der kalten Titration fünf Tropfen nicht überschreiten. In der Regel werden 1 bis 3 Tropfen benötigt. Eine Wiederholung des sauren Aufkochens mit Kühlzapfen und erneute Austitration in der Kälte ist in all den Fällen angezeigt, in denen mehr als fünf Tropfen „kalt" verbraucht wurden. Die Rosafarbe des Endpunktes bleibt im unverschlossenen Titrierkolben lange bestehen. Die Einstellung der Lauge erfolgt unter denselben Bedingungen.

Ich habe Herrn Dr. *W. Zimmermann* für all die Hilfe zu danken, die er mir bei der Abfassung dieser Arbeit hat zuteil werden lassen.

Zusammenfassung.

Die Feststellung des Äquivalenzpunktes einer mikroacidimetrischen Titration mit Phenolphthalein als Indikator in der Hitze ist unsicher. Es wird gezeigt, daß die Titration besser in der Kälte zu Ende geführt wird. Eine mögliche Fehlerquelle wird diskutiert.

Summary.

The determination of the equivalence point in a microacidimetric titration with phenolphtalein as indicator is not reliable in warm solutions.

* Große Wattepfropfen an den Enden der Natronasbestfüllung verhindern das Hineinfallen von Natronasbeststaub in die Titrierlösung.

It is shown that the titration is better carried to conclusion in the cold. A possible source of error is discussed.

Résumé.

L'établissement du point d'équivalence d'un titrage micro-acidimétrique avec la phénolphtaléine à chaud n'est pas sûr; on montre que le titrage est meilleur quand on le finit à froid. On discute une cause possible d'erreur.

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